sepmag®

Monitor

Biomagnetic Separation Monitoring & Management Software

User's Guide

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Welcome

Monitor is an electronic system for real-time tracing of optical changes in magnetic bead suspensions. Combined with the constant magnetic force of a **Sepmag** separator, the resulting graph offers detailed insight into the biomagnetic separation process dynamics.

The current version of the electronics and its associated software has been designed to help industrial and academic researchers develop new biomagnetic separation products and processes. Each change in the suspension (magnetic bead diameter or concentration, buffer composition, etc.) affects separation behavior.

Monitor makes it possible to measure multiple samples (up to 4) simultaneously and use reference curves for comparison. We hope these features and the built-in reports will be helpful and that they will improve the development of your biomagnetic separation process.

The **Monitor** software can be connected to the small tube adaptors for the **Sepmag® LAB** and **Sepmag® A** families, but also with the built-in electronics for plastic and glass bottles in the **Sepmag® A, N** and **Q** series.

This manual is divided into four sections. The first is an introduction to the software, which explains how it works, the basic concepts and what's new.

The second section tells you how to install the software on your computer and how to connect the **Sepmag®** hardware.

***Fig. 1**: **Sepmag®A200ml** with **Monitor** adaptor, connected to a laptop by USB.*

The third section is a manual for users with standard privileges that explains how to measure, print reports and check historical data.

The fourth section of the manual covers functions that are accessible only to users with administrator privileges (managing users, processes and hardware). Administrators also have access to ‘standard’ functions, so we recommend you read sections three and four before using the software.

At the end of the document you will find a glossary with a brief definition of the key words and concepts used in this manual.

1. INTRODUCTION: BASIC CONCEPTS

Monitor is an evolution of **Sepmag®**'s monitoring electronics, known as QCR, used by IVD companies for the Quality Control of biomagnetic separation production processes. Although QCR was originally intended for Production, the R&D departments in these companies also began to use the software and electronics for monitoring the samples at the lab level. These are two main differences between these customers and the QCR users: (a) they work with smaller volumes -they measure in milliliters, not in liters- and (b) their main interest is not in verifying batch-to-batch consistency, rather in comparing the differences between different samples. As result, our engineering team has developed (a) the new **Sepmag® LAB** and a family of adaptors/inserts for the **Sepmag® A** to accommodate small tubes and (b) **Monitor**, a new software interface to fulfill the specific need of industrial and academic researchers.

1.1 What is MONITOR?

Monitor is an electronic hardware, firmware and software system for measuring changes in opacity in a transparent/translucent vessel inserted in a **Sepmag®** biomagnetic separation system.

Monitor has been developed to give technicians and researchers a way of obtaining an objective measurement of the biomagnetic separation process. The goal is to replace the traditional determination of the separation time by visual estimation with a measurement of the opacity of the suspension throughout the ENTIRE PROCESS.

Monitor is composed of a cold lighting assembly of LEDs and an optical sensor. Both subsystems are controlled by an electronic card connected to a PC running Windows through a USB port. The software identifies the specific **Sepmag®** unit, records the data captured, generates printer-friendly reports, saves the historical data in a database and helps manage the steps in the biomagnetic separation process.

The hardware is supplied integrated into **Sepmag®** biomagnetic separation systems and/or independent insert (adaptor) for small tubesThe hardware is normally supplied integrated into **Sepmag®** Biomagnetic Separation Systems or its inserts.

1.2 Why should I monitor my biomagnetic separation processes?

The traditional way of checking whether a biomagnetic separation 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/Not OK signed by the person handling the vessel, with no supporting data. In the event of a quality issue with the product, it is not detected until a later stage, and there are no records 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 **Sepmag**, technicians have a record of its evolution during the process. Changes in opacity inside a **Sepmag** Biomagnetic Separation System 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 behaviors, so any deviations from the expected pattern serve as an early warning system.

The process is documented with printed reports for each stage. The acceptance parameter can be defined objectively, without subjective operator opinions. Moreover, the cumulative experimental data enables teams (technicians, researchers, and managers) to audit, analyze, review and improve the biomagnetic separation steps in their manufacturing processes.

1.3 What does MONITOR measure?

MONITOR measures change in transmitted light through the bottle. Using cold light (LED), the vessel is illuminated, and a sensor measures the transmitted light.

At the start of the process, when the suspension is homogenous, opacity is at its maximum level. When separation is complete, the remaining suspension is clear, and opacity is at its minimum level.

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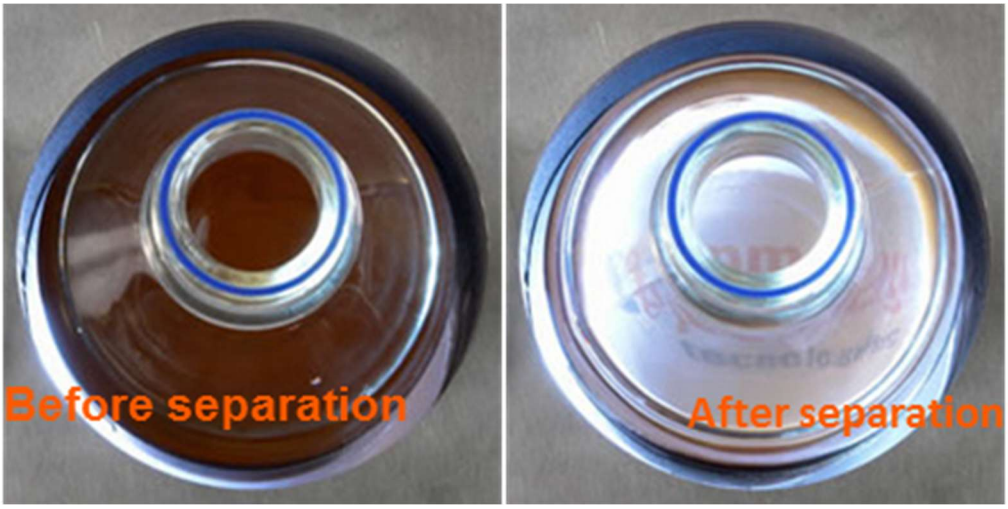

***Fig. 2**: Opacity of a typical magnetic bead suspension. Before separation, it is dark and homogenous. When separation is complete, the beads are left on the wall and the suspension becomes transparent.*

1.4 How does MONITOR characterize the biomagnetic separation process?

The typical biomagnetic separation curve has a sigmoidal shape. As discussed in several technical documents (www.sepmag.eu/resources/ebooks, www.sepmag.eu/blog), both the slope (defined by the dimensionless exponent *'p'*) and the separation time (t_{50}, expressed in seconds) depend on the buffer viscosity, the properties of the magnetic beads and the concentration. The biomagnetic separation conditions are homogenous and well known for each **Sepmag**, and both parameters can be used to define and validate each separation process.

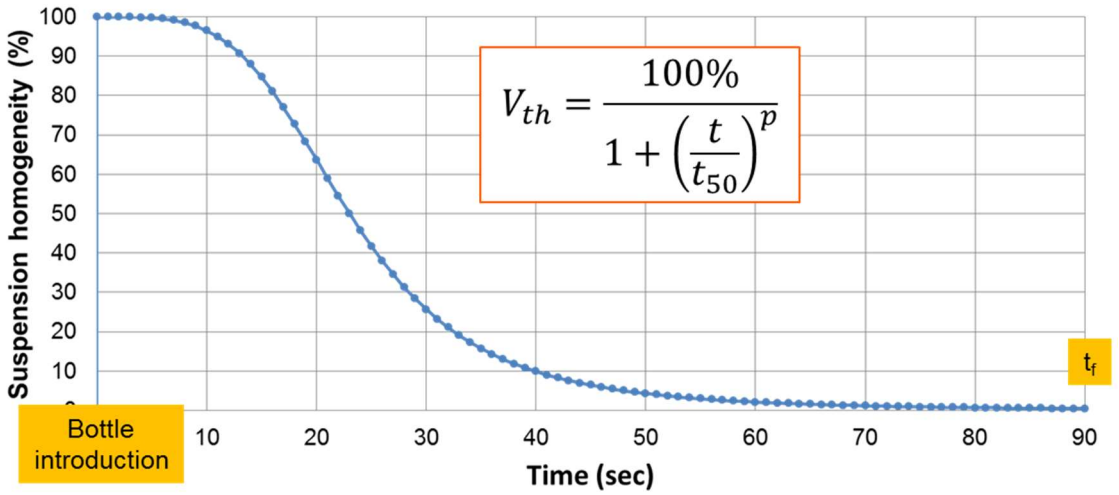

***Fig. 3**: Typical shape of biomagnetic separation curves measured using a **Sepmag**® System and **Monitor** software.*

1.5 What happens while the bottle is in the SEPMAG?

The **Monitor** measurement starts automatically when the sample is inserted into the device. As the magnetic force inside a **Sepmag®** is constant, the separation process starts with all the magnetic beads experiencing the same conditions. The opacity of the sample (maximum at the start when the suspension is homogeneous) changes and the sensors capture and record the value at a constant sample rate. When all the beads are separated, the sample transparency is maximum (minimum opacity), as only the buffer interferes with the light on the tube. Then the optical curve becomes stable and the user may stop the measurement.

1.6 Multi-sample measurement

An R&D process usually requires comparing several samples (for example: different beads, concentration, buffer compositions). That is why the adaptors (inserts) for small tubes enable several samples to be measured. Once one measurement is underway, the user can start an independent second one using an empty housing. Each of the measurements will run independently and can be stopped by the user at any time.

If the adaptor has additional empty positions, the process can be repeated until all the position are occupied. Once any of the measurements is completed and the sample has been removed, the empty position can be used again.

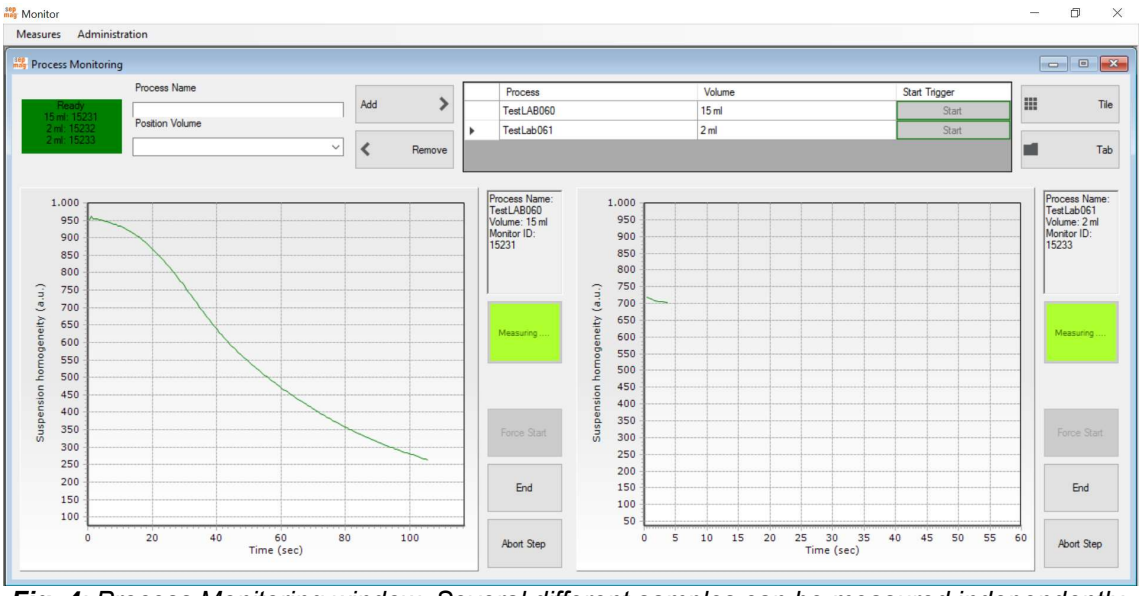

***Fig. 4**: Process Monitoring window. Several different samples can be measured independently.*

1.7 Individual working position identification

Each **Sepmag®** sample housing is identified with a specific ID. To avoid confusion and/or mistakes, the software first checks that you have connected the right hardware (each **Sepmag®** and adaptor has its own ID). Only the available positions are shown for selection.

Additionally, once you select the tube or bottle you plan to use, the software only allows the trigger in the corresponding housing. If you select a 2-ml tube, **Monitor** would only trigger the measurement if you place the sample in a 2-ml housing, and not, for example, if you introduce it in a 15-ml housing.

1.8 Measurement parameter management

Unlike in Production processes, the R&D samples have a much broader range of optical and magnetic characteristics. For that reason, the **Monitor** software enables the user to change two basic measurement parameters.

The first parameter is the trigger level. For concentrated samples, the initial opacity is usually high, there is some contrast between the empty sample holder and when the sample is in. The higher the trigger level, the lower the possibility of having a false measurement start. However, when the sample is diluted, the optical contrast decreases, so the trigger level must be lowered. Due the wide range of sample properties in an R&D department, the trigger default values can be adjusted by the user with Administrator privileges.

A similar problem can arise with the sample rate. For samples that separate quickly, the default 200 ms sample rate would be a good option. But for an R&D project involving small nanoparticles, the separation time can be several hours, and the sample rate may need to be adjusted to several seconds. As for the trigger level, the software Administrator can adjust the sample rate.

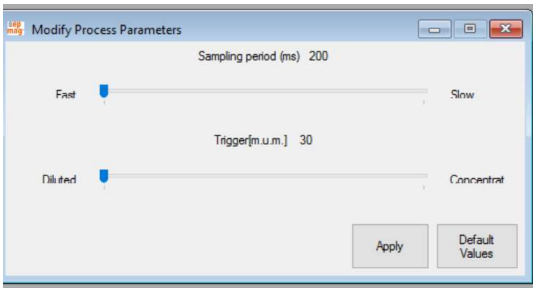

***Fig. 5**: Measurement Process Parameters.*

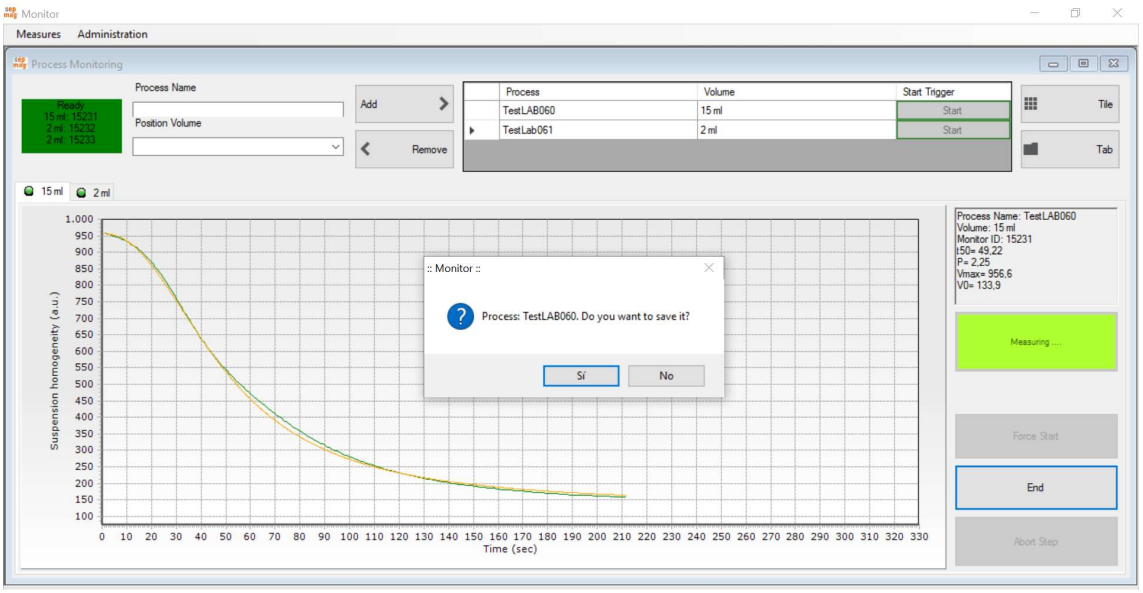

***Fig. 6**: Experimental curve (green) and fitting curve (orange). Fitting results are shown in the box on the right (t_{50th}, p_{th}).*

1.9 User categories: standard and administrator

The program distinguishes between two different user categories:

i) Users with ***administrator*** privileges and

ii) ***Standard*** users.

Standard users have access to the **Measures** menu, which includes the options **Process Monitoring** (measuring) and **Retrieve Data** (consult data from older processes, generate and print reports, export measurements). Section 3 describes these functions in detail.

Administrators also have access to the 'Administration' menu. This menu includes the **Add/Modify Users** option (define new users, modify privileges), **Add/Manage Folders** section (organize the measurement files in folders), **Modify Process Parameters** (select trigger level and sample rate), **Add/Modify Devices** (for including new Sepmag hardware) and **Audit Trail** (a log of changes made).

Changes made by the administrator are recorded in the audit trail. This log is also accessible in the administration menu, but it is not editable.

The following section explains how to install the hardware and software and the functions accessible to standard and administrator users.

2. INSTALLATION

The previous section described the **Monitor** software features. This section describes how to set up your system. It indicates the computer requirements, how to install the software, the hardware supplied materials list and how to connect the **Sepmag®** equipment.

2.1 System Requirements

A PC computer with an available USB port for connecting the **Sepmag®** hardware is required. If a laptop computer is used, we recommended that the computer remain plugged into the power source while the **Monitor** application is running to avoid a power failure that could lead to a loss of process information.

The **Monitor** software is designed to run on **Windows 10** or later versions of Windows. The minimum requirements are a Core i5 or i7 processor, 8 GB RAM and an available USB port.

2.2 List of materials supplied with the SEPMAG

The Monitor electronics include:

1. **Monitor** hardware elements, including the **Sepmag®** adaptors: **MLABxxx** insert for **Sepmag® LAB** or **MAxxx** for **Sepmag® A**. The hardware integrated in the **Sepmag® A**, **N** and **Q** is also compatible with **Monitor** software.
2. Cable to connect the QCR device to a computer via a USB port.
3. **Monitor** data acquisition software (the software is supplied either as a hard copy -flash memory- or as a link to the downloadable file).

2.3 Unpacking, assembling and installation guidelines

To guarantee correct performance of the **Monitor** system, please follow these steps:

1. Find the micro-USB port on the **Sepmag®** system.
2. Check that your computer meets the system requirements specified in Section 2.1 and is compatible with the **Monitor** system.
3. Make sure the computer has a free USB port to connect to the **Sepmag®** and that the USB port is compatible.
4. Install the **Monitor** software on your computer (see section 2.4)
5. Connect the hardware according to the instructions in section 2.5. The system is now ready to start operating (sections 3 and 4).

2.4 Software Installation

To run the software installation, execute the setup file (*Monitor_v3.2.x.exe*) provided to you either as a hard copy or through an internet link.

The setup program will guide you through the steps required to install the software on your computer **(Fig. 7).** It will create a new directory containing all the necessary files in the selected location on your computer**.** When the installation process is complete, a shortcut to the program will automatically appear on the computer desktop.

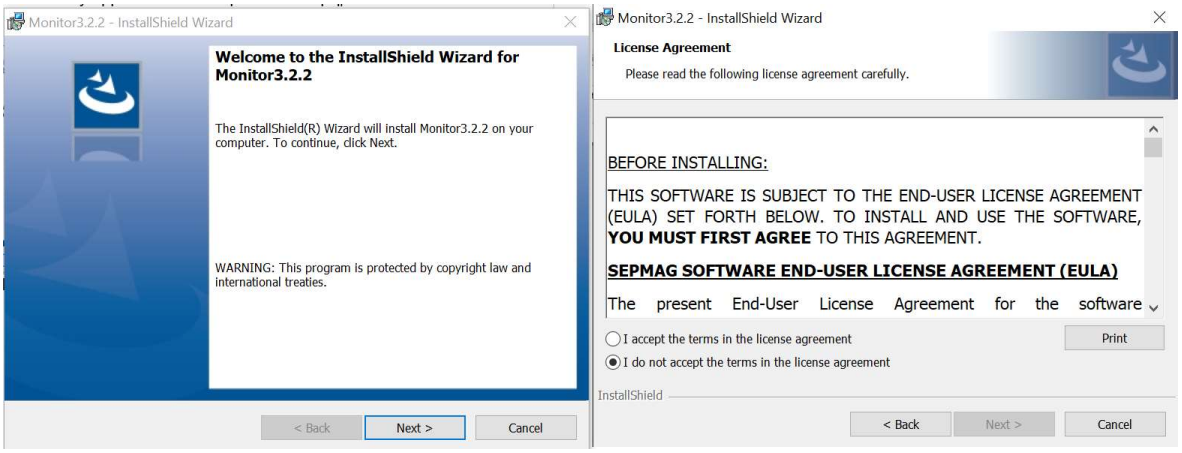

Fig. 7: **Monitor** software installation process.

2.5 Hardware – Software interface

Once you have installed the software and assembled the equipment, connect the **Sepmag**® to the computer using the USB cable provided **(Fig. 8):**

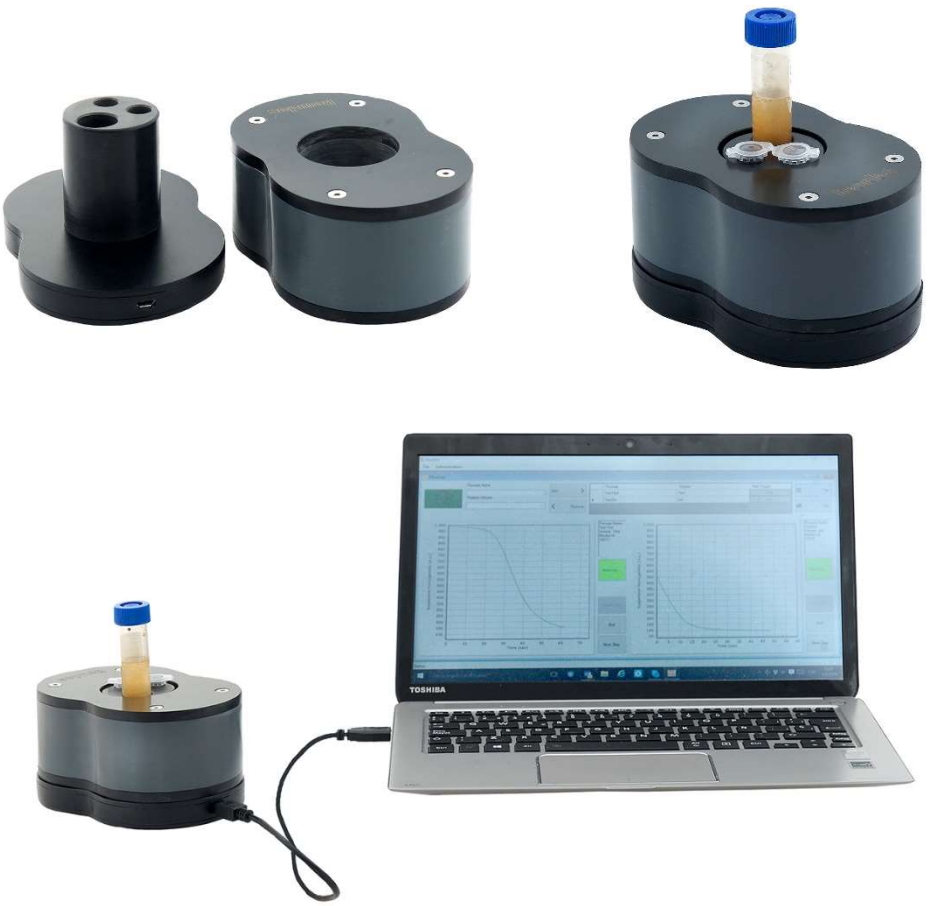

Fig. 8: Installing the **Sepmag**® equipped with **Monitor** electronics.

A. Have the USB cable provided ready to connect the computer and the **Monitor** hardware for data acquisition.

B. Connect one end of the USB cable to the port on the **Monitor** device.

C. Connect the USB end of the cable to the USB port on the computer and start running the **Monitor** software to monitor your process (See sections 3 and 4 for details).

2.6 MONITOR database backup

All processes saved by the different users are stored in the **Monitor** database. Every time the user exits the program, a backup copy of the database is created and saved by the program in the folder:

file:///Volumes/C/Users/Public/Documents/Sepmag/MONITOR/.

The database (*SepmagMonitorDDBBv2.mdb _lastCopy*) is a backup copy of the program database (*SepmagMonitorDDBBv2.mdb*). It is important to keep a copy of this backup file in a safe place, because the original database will be lost if the program must be reinstalled on the computer.

It is very important to keep a copy of the** SepmagMonitorDDBBv2.mdb **back up file in a safe place.

3. RUNNING THE QUALITANCE SOFTWARE AS A STANDARD USER

Start the application by double-clicking the shortcut represented by the **Sepmag®** logo, which appears on the computer desktop after the set-up process is complete and the software has been correctly installed on the computer.

When you start the program, a *Login* dialog window pops up. Users must enter a Username and Password to start a session **(Fig. 9).**

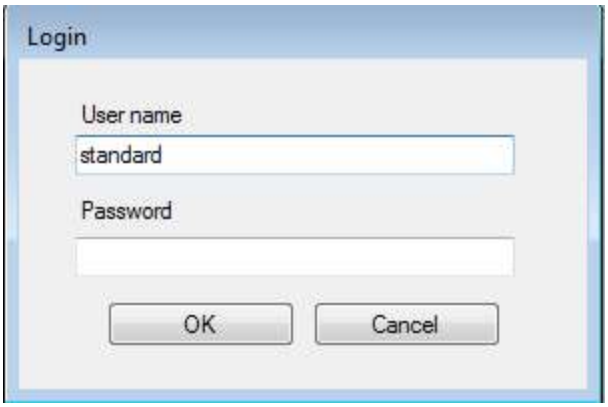

***Fig. 9**: Initial dialog window for user identification. Users must log in to start a session. The program requires a username and password.*

When the program is used for the first time, the default username is *standard* and the default password is *standard.* This default configuration has *standard* privileges.

A user with standard privileges has access to the *Measures* menu, to execute the defined product process through *Process Monitoring* (section 3.1), as well as access to previous measurements through *Retrieve Data* (section 3.2).

The *Logout* option takes you back to the *Login* menu, allowing a different user to log in without exiting the application.

Exit closes the program and returns to Windows. You can also exit the program by closing the main menu window (by clicking the X in the top right-hand corner).

***Fig. 10**: User interface for the main menu of the program. To exit the program, either select Exit in the Measures menu or simply close the main menu window.*

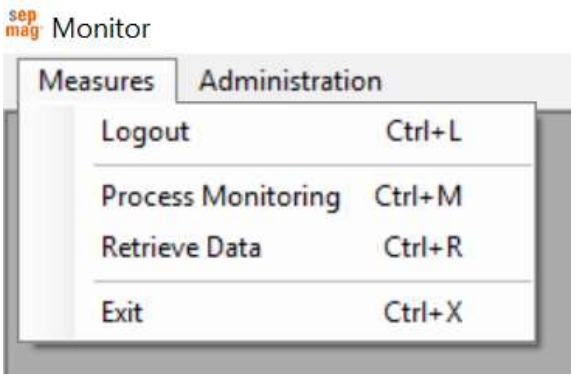

***Fig. 11**: Initial dialog window for users with standard privileges. The Process Monitoring option gives access to the measurement menu and Retrieve Data enables you to consult existing measurements*

3.1 Measures: Process Monitoring

Click the ***Process Monitoring*** option. If no **Sepmag®** system is connected, the message at the top left is shown in red and reads ***Not Ready***.

If a **Sepmag®** system is connected, the message is green and reads ***Ready***, providing the list of available positions and sensor IDs.

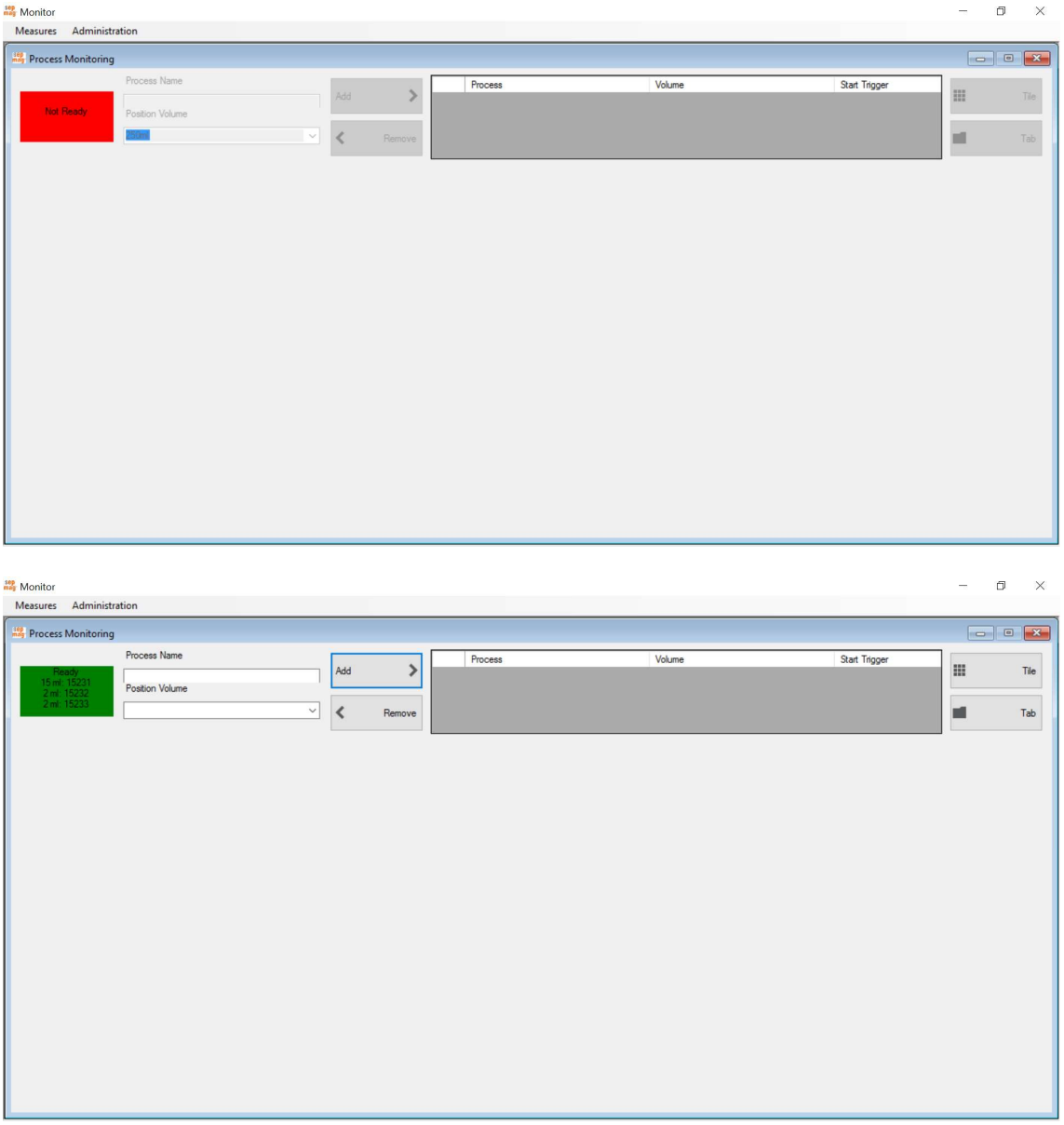

***Fig. 12**: Process Monitoring window when no **Sepmag®** device is detected (above) and when a **Sepmag®** device is correctly connected via USB port to the computer running the **Monitor** software (below).*

To start a measurement, the process name must be entered manually in **Process Name**. The software checks the database and issues a warning if the process name already exists. Duplicate names are not allowed.

Then, select the volume from the **Position Volume** list and click **Add**. The list displays all available positions. If a position is measuring, it does not appear on the list.

The table on the right shows the list of measurements ready to start. When ready, click **Start Step**. Then a ***Waiting*** message flashes on an orange background. **<u>This means that the system is ready and waiting for the tube/bottle to be inserted.</u>**

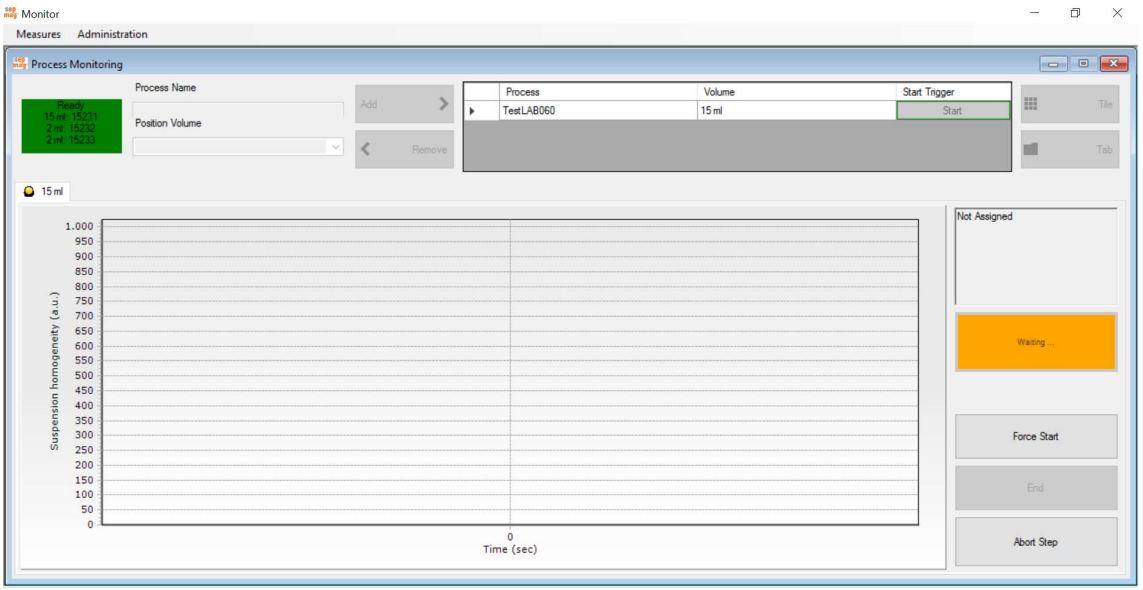

***Fig. 13**: System ready to take a new measurement. The Waiting message indicates that **Monitor** is waiting for the sample to be introduced.*

Place the sample in the **Sepmag®** system and the measurement will start automatically (**Force Start** can be used in rare cases in which the trigger fails to activate

the measurement. See also section 4.3 to learn how to modify the trigger values.). The message will change to ***Measuring*** with a light-green background.

Additional measurements can be launched in the available positions while the current process is measuring. Clicking the **Tab** or **Tile** buttons (upper right-hand corner of the screen) enables you to view a single measurement or all active processes.

To finish one of the measurements, click **End** or **Abort Step**. When you click End, the software then asks whether to save the measurement (if the answer is 'no', it asks you to confirm your answer).

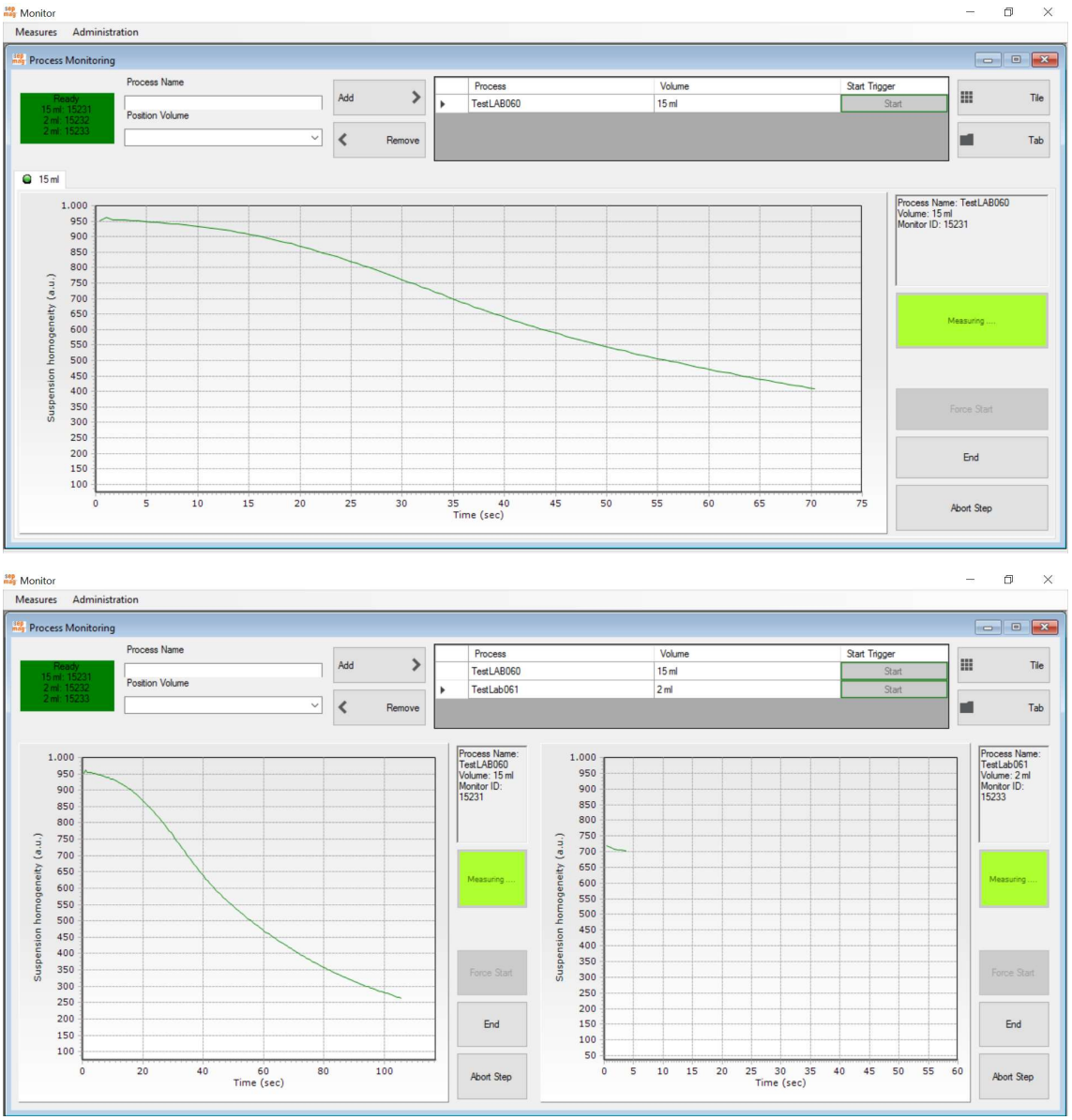

***Fig. 14**: Experimental curve (green) shown in Tab (top) and Tile (bottom) view.*

You can then monitor the other measurement processes, start a new measurement or exit the measures window.

As explained in the following section, all data is accessible in the *Retrieve Data* window, through the *Measures* menu.

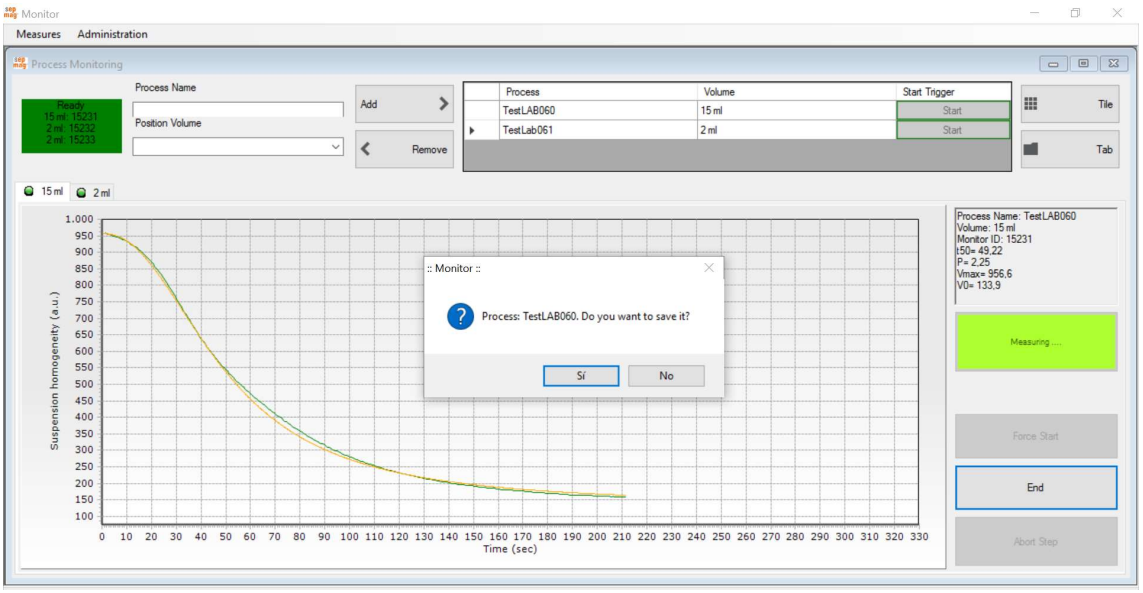

Fig. 15: *Measurement complete. The green curve is the experimental curve, the orange is the fitting to a sigmoidal. The fitting results are displayed in a box over the 'Measuring' message.*

3.2 Access to recorded data: Retrieve Data

The main ***Measures*** menu gives Standard users access to existing measurements. It prints the reports of the completed processes and compares different samples.

To access these features, click the **Retrieve Data** option (accessible even if no **Sepmag®** system is connected to the computer).

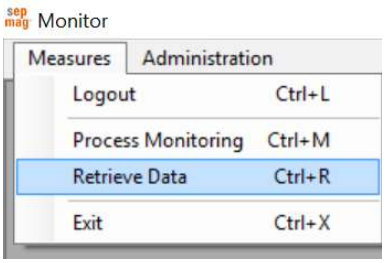

Fig. 16: *Measures menu. The Retrieve Data option displays existing measurements.*

The *Retrieve Data* window displays the measurement processes. The top left-hand table shows the folders. The user may select which samples are to be shown in the **Selected Folder Processes** (see section 4.2 to learn how to create and manage folders). When you select one of the sample names displayed in the center table and click **Add**, the name also appears in the table on the right (**Processes Selected**), and the biomagnetic separation curve is displayed on the screen. The curve can be removed from the graph by clicking the **Graph** box. To remove the sample from the **Processes Selected** table, select it and click **Remove.**

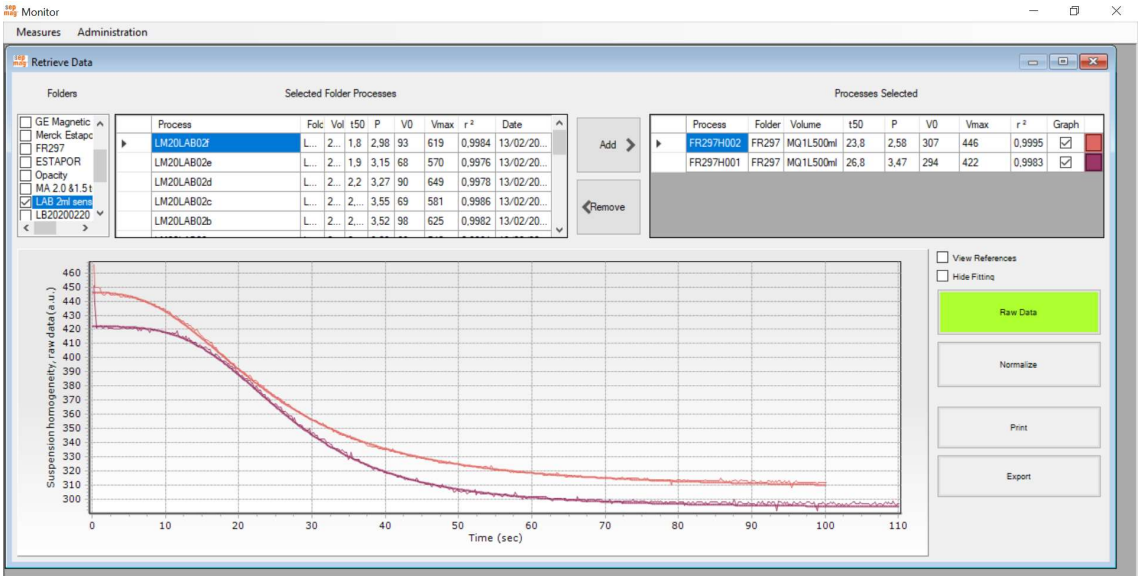

Fig. 17: *Process window. The samples from the selected Folders are displayed in the central table. The samples selected in this table are displayed in the table on the right and, if the Graph box is selected (default option), displayed in the graph.*

The default graph shows the **Raw Data** (i.e., the values from the sensor output) and the Sigmoidal fitting of the data (see section 1.4 for details on the fitted curve). The fitting can be hidden by selecting the **Hide Fitting** check box. To compare curves from different samples or different opacities (for example, different concentrations), the samples can also be displayed normalized by clicking **Normalize**. The minimum (0%) and maximum (100%) values are obtained from the fitting. Finally, the graph can also show the reference curve for the selected folders by selecting the **View References** check box.

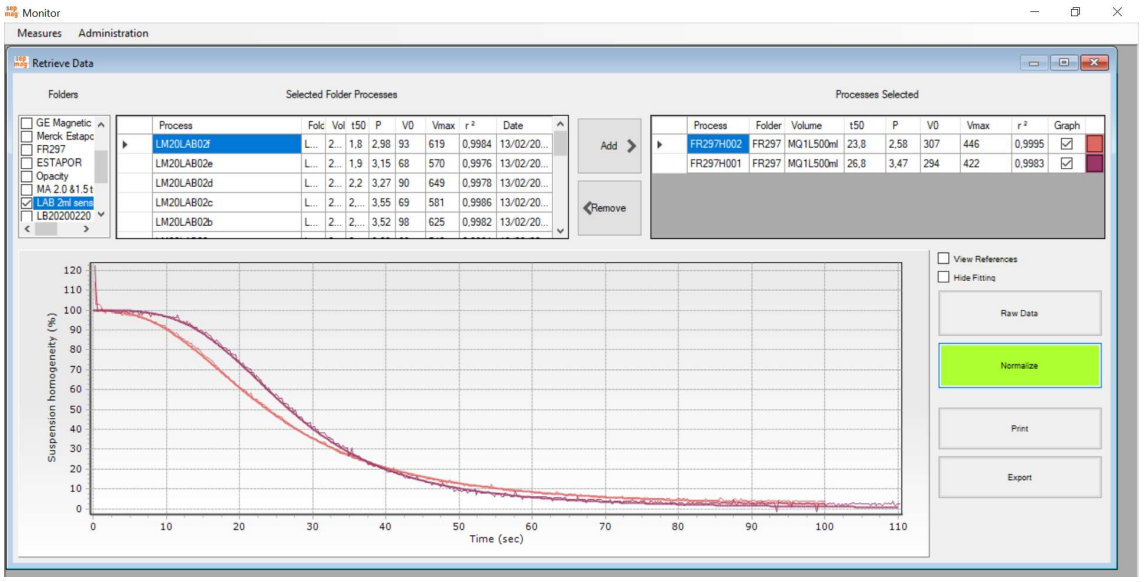

Fig. 18: *Process window. The Curves with 'graph' ON are shown normalized.*

Once the graph is complete with the selected curves, it is possible to select two additional features:

Print generates a standard printer-friendly report (a new window pops up with the document).

Export generates a data file with all the recorded data. The file can be in *.xls (Excel) or *.txt format. NOTE: the exported data also includes the values between 'tf' and the bottle extraction –or Force End–.

3.3 Improving the data fitting

The optical changes of the suspension when it is inside the **Sepmag®** (i.e. when a constant magnetic force is applied) can usually be fitted to a sigmoidal curve. To test the quality of the fitting, the software provides the coefficient of determination r^2.

If r^2<0.99, the obtained fitting values (t50, p, V0, Vmax, t99) should be critically revised.

A bad correlation coefficient can be caused by a bad fitting (the algorithm can't converge to a good curve) or because the separation curve doesn't have a sigmoidal behavior.

In **Monitor**, you may modify these fitting parameters at the Measuring/Retrieve Data window. At the Selected Process table, you should click the right square indicating the color of the curve and you access to a new window where you may play with the parameters. Once 'save', they will be filed in the database.

The 'Fitting Help' provides an additional explanation about how the different parameters may be used to improve the fitting quality (see also Annex: Sigmoidal curve fitting troubleshooting)

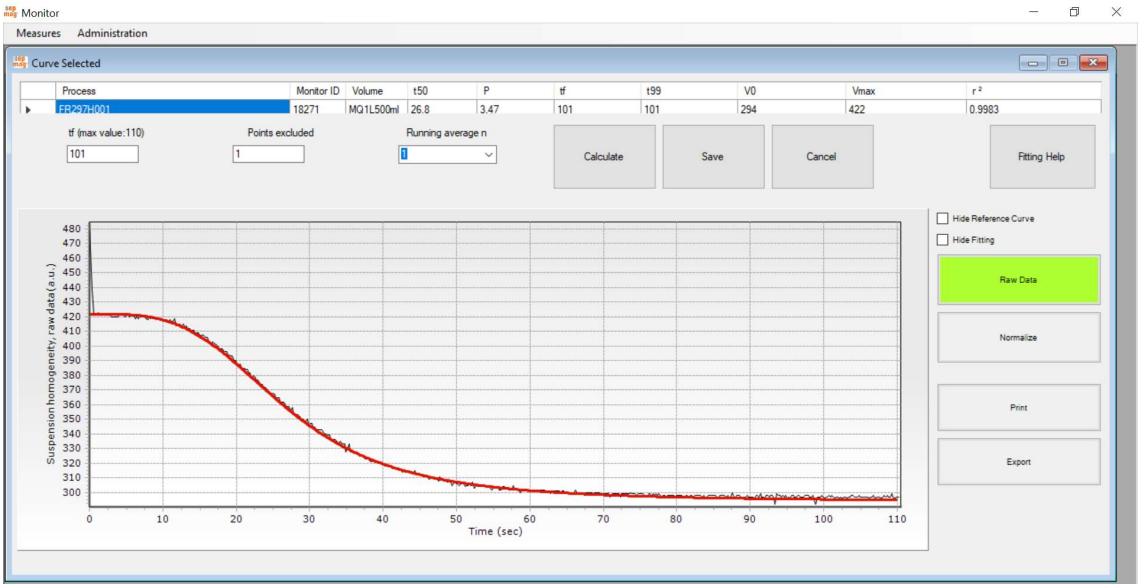

***Fig. 19**: Selected curve window*

In addition to the standard function, **Monitor** has functions accessible only to users with administration privileges. The fourth section of the manual describes these additional features.

4. RUNNING THE MONITOR SOFTWARE AS AN ADMINISTRATOR USER

Monitor has two user categories. Standard users can use the program to take measurements, print reports and consult previous measurements. All these functions are accessible through the Measures menu. Administrators have access to both the **Measures** and **Administration** menus, where they can set up new **Sepmag®** hardware, add users, create folders, select curves as reference and modify the measurement parameters.

The Administration menu functions can also be executed when no **Sepmag®** hardware is connected to the computer.

This section describes the functions available only to **Administrators**. For Standard functions, please see Section 3 (*Running the Monitor software as a Standard User*).

Start the application by double-clicking the shortcut represented by the **Sepmag®** logo, which appears on the computer desktop after the set-up process is complete and the software has been correctly installed on the computer.

When you launch the program, a **Login** dialog window pops up and asks you for your **Username** and **Password** to start a session (Fig. 20).

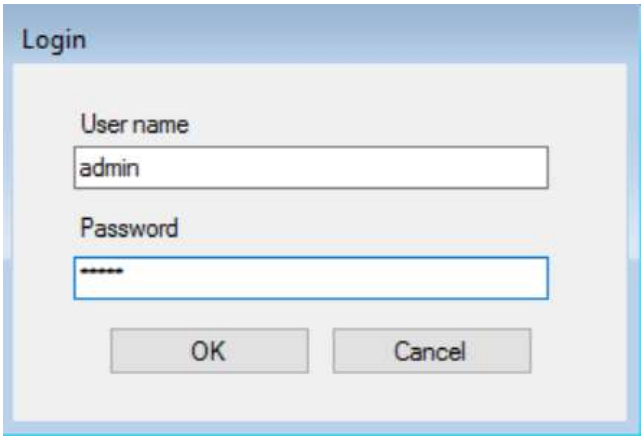

Fig. 20: *Initial dialog window for user identification. Users must log in to start a session. The program asks for a username and password.*

When you use the program for the first time, the default **Username** is *admin* and the default **Password** is *admin*. This default configuration has administrator privileges. (NOTE: Because the admin username and password are published in the manual, it is strongly recommended that you change the password. See section 4.1 for instructions).

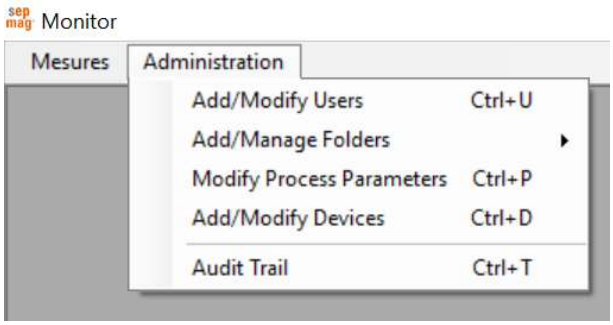

Fig. 21: *Administration menu.*

In addition to the **Measures** menu, the **Administration** menu appears in the top left-hand part of the window. Click to see the four options:

- **Add/Modify Users:** This is where you create and edit user accounts and privileges.
- **Add/Manage Folders**: This is where you define and edit Folders to organize your samples and where you select the reference curve for each Folder.
- **Modify Process Parameters**: Enables you to change the trigger level and the sampling rate.
- **Add/Modify Devices**: This is where the **Monitor** software assigns a name and ID to each device and measuring position. By default, the installer includes the values for the **Sepmag®** systems supplied with the software.

The following sections provide details on these four options.

4.1 Create or edit users: Add/Modify Users menu

When you click **Add/Modify Users** in the **Administration** menu, the user administration window opens. The table at the top lists the **Username**, **Password** and whether the user has **Administrator** privileges.

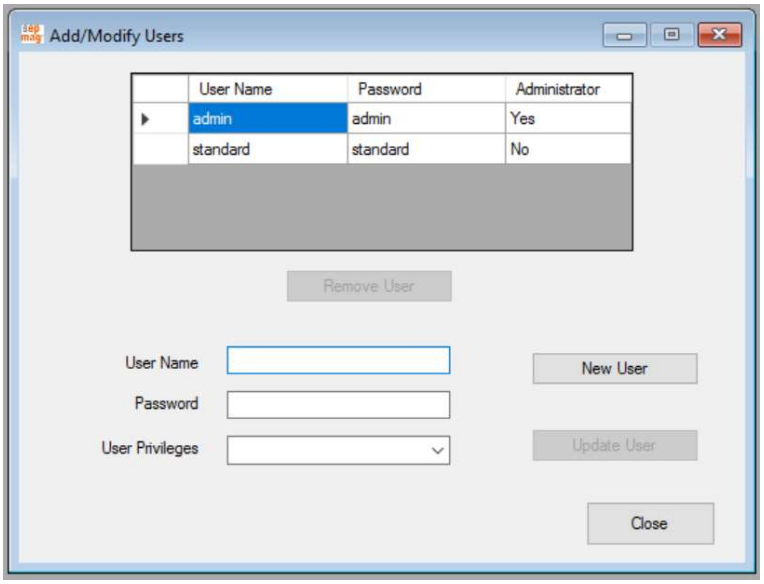

***Fig. 22**: Access the Add/Modify Users window by clicking Add/Modify Users in the Administration menu.*

To create a New User, enter the Username, Password and User Privileges and click **New User**.

To edit/delete an existing user, click the table row with the user you want to modify. The **Remove User** and **Update User** options are displayed.

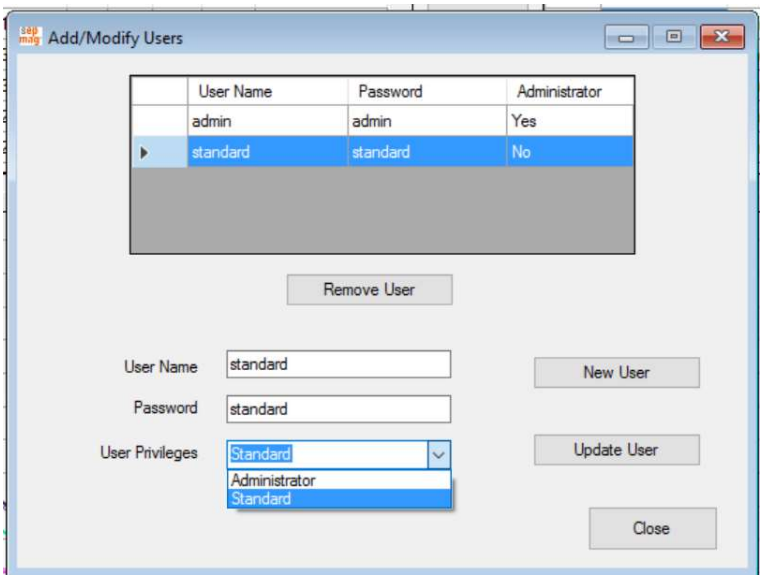

***Fig. 23**: User administration window.*

The Username, Password and/or User Privileges can be then edited. Click **Update User**. A pop-up window will be displayed asking you to confirm the changes.

Click **Remove User** to delete the account (a warning message appears and asks for confirmation before deleting the user). There must always be at least one user with administrator privileges. The software does not allow you to delete the last administrator.

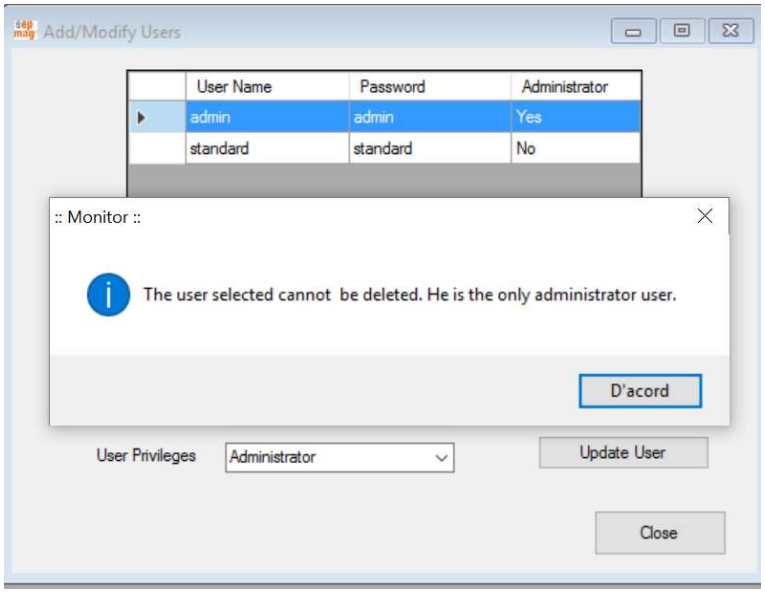

***Fig. 24**: Add/Modify Users window. This warning is displayed if you attempt to remove the last administrator user.*

To return to the **Administration** menu, click **Close** or close the Add/Modify Users window.

4.2 Organize and classify your measurements: Add/Manage Folders

After just a few measurements, the number of biomagnetic separation process measurements would be large enough to be difficult to manage in a single folder. **Monitor** allows the users to generate folders to classify the samples, as well as to select some of the measurements as references for comparison with the new measurements.

The **Add/Mange Folders** option is included in the **Administration** menu. When you click it, two options are shown: **Add New Folder** for creating a new Folder and **Manage Folders/Files** to assign the curves and references to the existent folders.

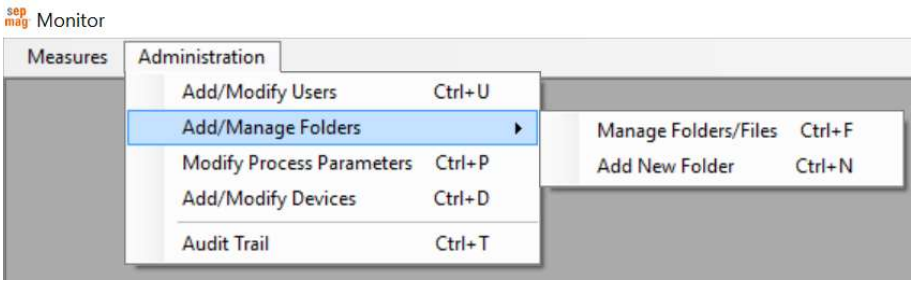

***Fig. 25**: Add/Manage Folders menu*

4.2.1 Creating new folders: Add New Folder

When you click **Add New Folder**, the **Add New Folder** window opens. The existing folders (if any) will be shown in the **Folders List** table on the left.

To create a new Folder, simply enter a new **Folder Name** in the box, as well as the description in the **Folder Description**. When you click **Add**, the folder appears on the **Folders List**.

The list will always include the **Processes Trash** folder. You may place the measurements you want to discard there so that it no longer appears when you click **All** in the Open Process window. You can retrieve these sample results in the future by moving them out of this folder (as described in the next section).

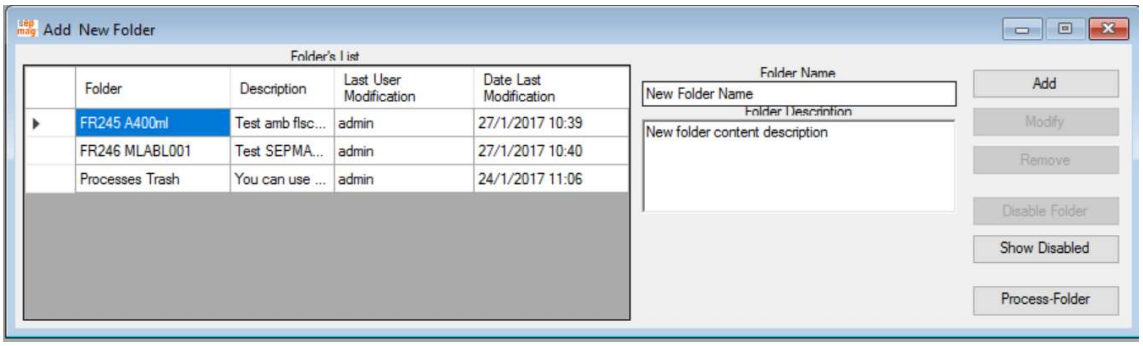

***Fig. 26**: Add New Folder window.*

In this window, you may also select one of the folders listed on the **Folders List**. Then several new options are highlighted:

- **Modify** enables you to change the name or the description of the selected folder.
- **Remove** deletes the selected folder. If the selected folder includes curves, it cannot be deleted. You will need to move all sample measurements out of the folder before you delete it.

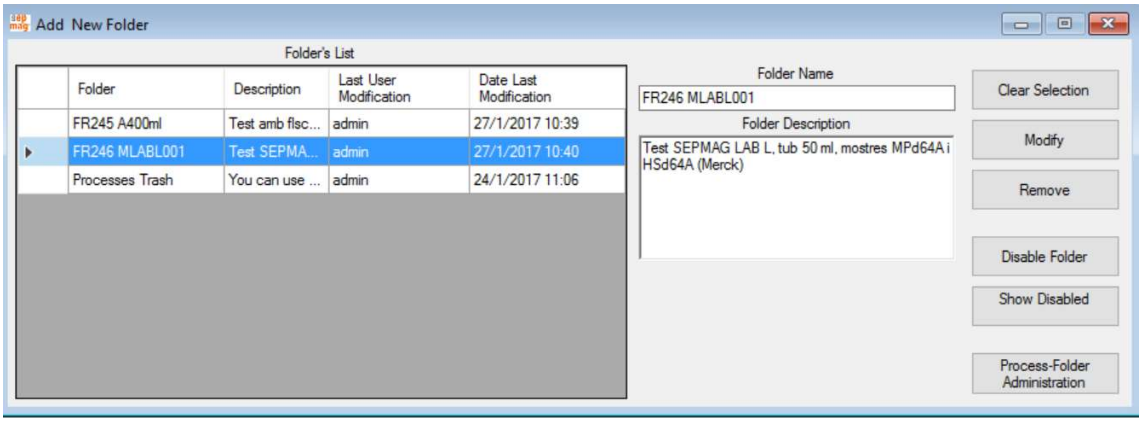

***Fig. 27**: Add New Folder window when one of the folders is selected.*

- **Disable Folder** will hide the folder and the assigned curves. The Folder Name disappears from the Folder List and the assigned sample measurements will not appear when you use the options in the **Measures** menu.
- **Show Disabled** will show the disabled folders. Selecting one of the disabled folders will enable you to click **Enable Folder** and restore it.

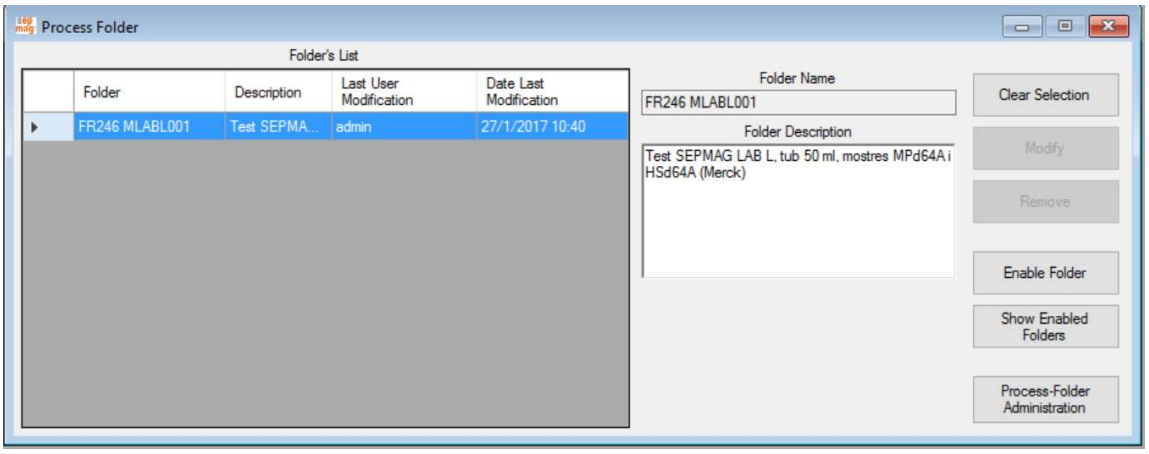

***Fig. 28**: Disabled Process Folder window.*

- **Process-Folder Administration** is a shortcut to the window where sample measurements can be managed and assigned as references.

4.2.2 Organizing sample measurements: Manage Folders/Files

The Manage Folders/Files window is where you can organize the sample measurements, assigning each one to a different Folder. You can reach this window in the **Administration/Add/Manage Folders/Manage Folders/File** menu, or directly in the **Add New Folder** window by clicking the **Process-Folder Administration** option.

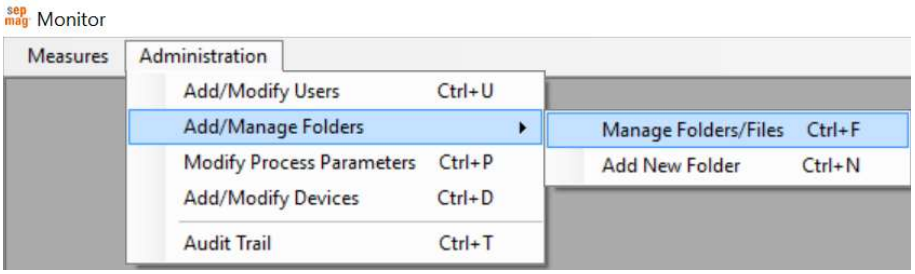

***Fig. 29**: Folders menu.*

When you access this window, the list of the **No linked** sample measurements appear in the **Selected Folder Processes** (left table) and in the Graph below. By clicking the box of any other folder, the sample measurement stored in it appears in the **Selected Folder Processes** table and in the Graph. By default, the Raw Data and the fitting are displayed. Changing the option to **Normalize** and/or **Hide Fitting** requires simply clicking the option on the screen.

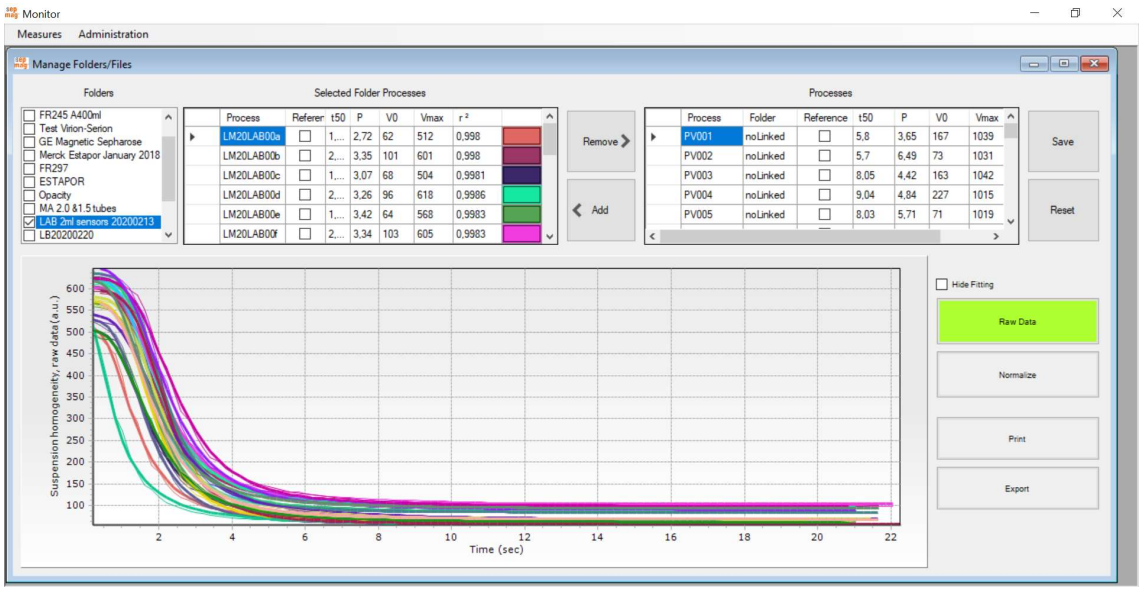

***Fig. 30**: Default view of the Manage Folders/Files window. Before you select a specific folder, the **No linked** samples are displayed as Selected.*

When a specific folder is selected, the **Processes** table (right) shows the **No linked** process. You may then select one of the **No linked** processes and assign it to the

selected folder by clicking **Add**. The program will ask for confirmation. If you click 'Yes', the new sample measurement will be displayed in the graph. To remove a sample measurement from the selected folder, the process is similar: select the sample measurement in **'Selected Folder Processes** and click **Remove**. You will be asked for confirmation. If you confirm, the graph disappears from the graph and the sample measurement moves to the **Processes** table, to be assigned as **No linked**.

You can move several graphs to/from the selected folder. When you finish the changes, you must click **Save** to file the changes. If you want to discard the changes, you can click **Restore**. The system will keep the sample measurements assigned as they were the last time you clicked **Save**.

If you want to move a sample measurement located in one folder to a different folder or to **Processes Trash**, you must first un-assign it (moving it to **No linked**) and then move it from **No linked** to the final destination. Remember that you need to click **Save** before you change the **Selected Folder** or **Exit** the window to file the changes.

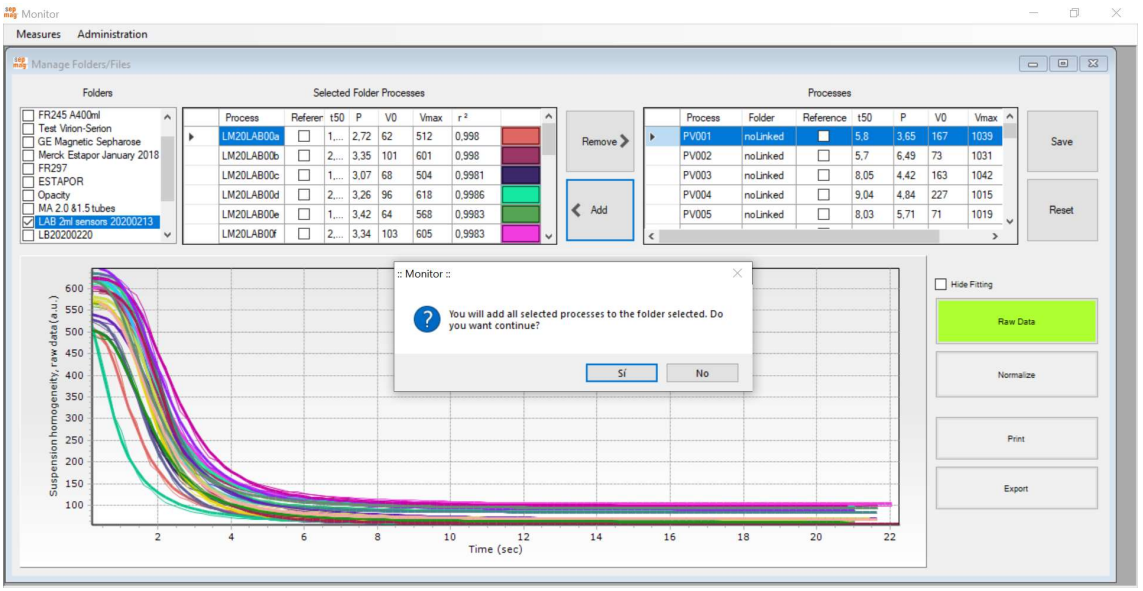

***Fig. 31**: Moving a sample measurement to a specific folder. The sample must be in the 'no linked' folder to be assigned to the selected folder. The changes will not be filed until you click Save.*

4.3 Changing the sampling rate and trigger level: Modify Process Parameters

Unlike in Production processes, the R&D samples have a much broader range of optical and magnetic characteristics. For that reason, the **Monitor** software enables the user to change two basic measurement parameters.

The first parameter is the trigger level. For concentrated samples, the initial opacity is usually high, there is some contrast between the empty sample holder and when the sample is in. The higher the trigger level, the lower the possibility of having a false measurement start. However, when the sample is diluted, the optical contrast decreases, so the trigger level must be lowered. Due the wide range of sample properties in an R&D department, the trigger default values can be adjusted by the user with Administrator privileges.

A similar problem can arise with the sample rate. For samples that separate quickly, the default 200 ms sample rate would be a good option. But for an R&D project involving small nanoparticles, the separation time can be several hours, and the sample rate may need to be adjusted to several seconds. As for the trigger level, the software Administrator can adjust the sample rate.

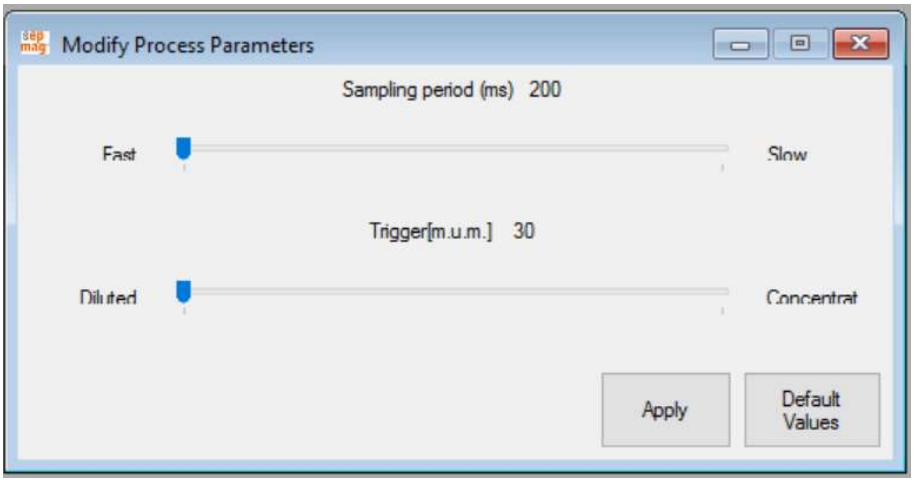

***Fig. 32**: Modify Process Parameters window. Both the sampling and the trigger level may be modified*

4.4 Connecting a new SEPMAG® to the computer: Add/Modify Devices

When a new **Sepmag®** device with monitoring electronics is received, a custom **Monitor** software installer is provided. In Add/Modify Devices, a table shows the name of the different volume positions and the individual identification number.

When you purchase additional **Sepmag®** devices, you will need to add the name of the device and its electronic ID. This allows the **Monitor** software to identify each individual measuring position.

To add the new **Sepmag®** hardware, simply enter the volume of the new measuring positions, the ID number (provided with the Technical Datasheet) and click **New Pair**.

If necessary, the values can be edited by selecting a row in the table (the Update Pair button is displayed), modify the values and click **Update Pair**.

To return to the Administration menu, click **Close** or close the User Administration window.

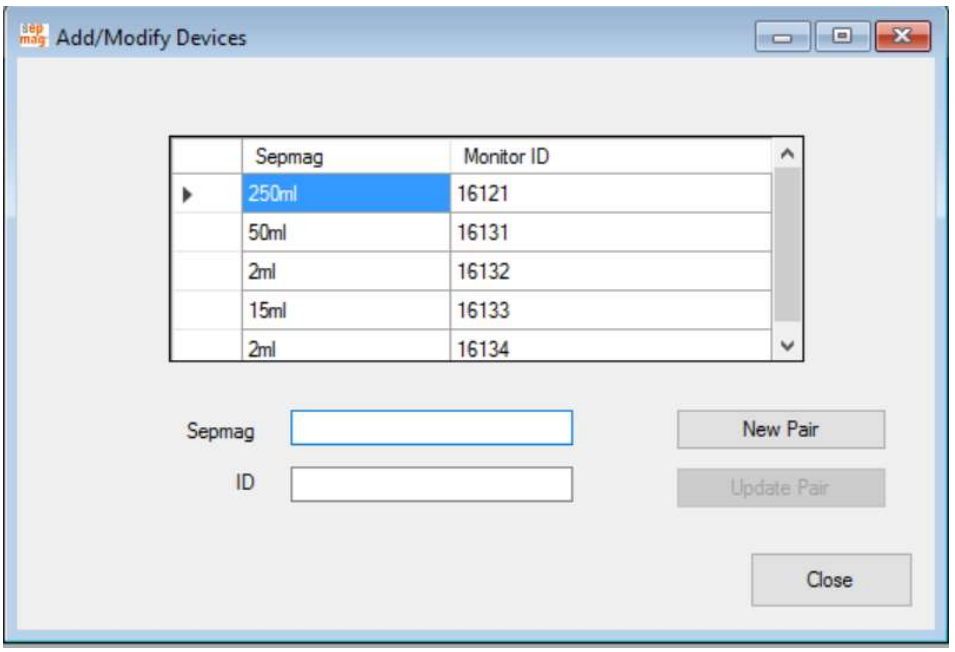

***Fig. 33**: Add/Modify Devices window. This shows the names of the measuring positions that can be connected and their electronic ID numbers.*

4.5 Audit Trail

Changes made by Administrators affect measurement records directly. Adding/modifying new users and **Sepmag®** devices and changing the measurement parameters has an impact on process reporting. The way we organize the different sample measurements, and the selection of the references may also be important for the results analysis.

Because this, all changes made in the Administration menu are logged in the **Audit Trail**. This provides a record of the changes for future Audits.

To access to the Audit records, go to the **Administration** menu and click **Audit Trail**. Select the start and end dates to display all changes made during that period.

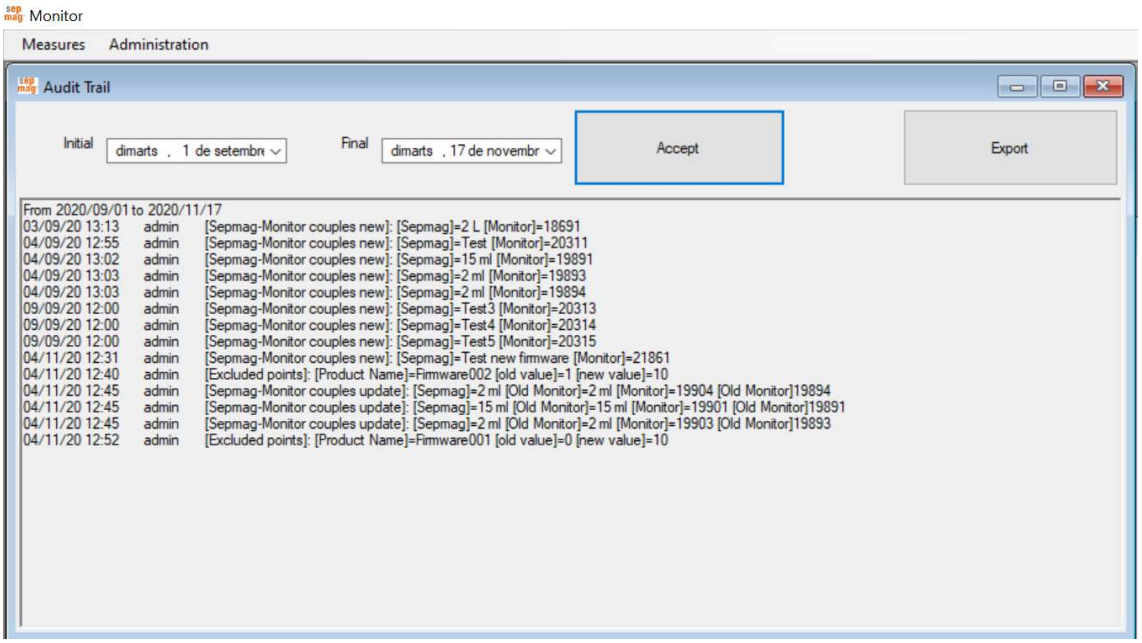

***Fig. 34**: Audit Trail window. Selecting the start and end dates shows all changes made using the Administration menu functions.*

GLOSSARY

Add/Manage Folders: Monitor menu for creating and managing folders and assigning measures to them**.**

Add/Modify Devices: Monitor menu for managing the **Sepmag®** devices connected to the computer.

Add/Modify Users: Monitor menu for managing the users and their privileges.

Administrator (or administration user): User who, in addition to the functions accessible to a *standard user*, can set up new **Sepmag®** hardware, add users, generate/modify folders and modify the process measurement parameters.

Audit Trail: Log file that records all changes made by the administrator in the Add/Modify Users, Folders, Process Parameters or Devices menus.

Experimental curve: Results of the acquisition of the optical opacity (to cold white light) while the vessel is in the **Sepmag®**.

Fitted curve: Sigmoidal curve showing the best approximation to the experimental curve using the less square methods. The parameters t_{50} and p are obtained from this fitting.

Measurement: Acquisition of optical opacity (to cold white light) while the vessel is in the **Sepmag®**.

Modify Process Parameters: Monitor menu for modifying the Sample Period and/or the Trigger Level.

Monitor: Software for measuring the biomagnetic separation process executed in **Sepmag®** Systems and managing the results.

Monitoring hardware: Electronic hardware and firmware that measures changes in opacity of a transparent/translucid vessel inserted in a **Sepmag®**.

p: Exponent of the Sigmoidal curve

Process Monitoring: Monitor Menu for taking new measurements.

Points excluded: Number of data points not used for the fitting at the beginning and the end of the measurement to eliminate the noise of the introduction/extraction of the bottle. The default value is 1.

Reference curve: Curve selected in a specific folder that is displayed as reference for comparison.

Retrieve Data: Monitor menu for retrieving measurements, printing reports and comparing curves.

Running average n: The number of data points used for the calculated the running average (smooth/filter noisy data). The average is calculated using the (n-1)/2 data before and after the central value. The default value is 1.

Sampling period: Time between two consecutive opacity measurements. The default value is 200 ms.

Sepmag®: Advanced Biomagnetic Separation System characterized by generating homogenous radial force in the bore.

Sigmoidal curve: Mathematical function defined as

$$V(t) = V_{min} + \frac{V_{max} - V_{min}}{1 + \left(\frac{t}{t_{50}}\right)^{p}}$$

Standard user: User authorized to perform measurements, print reports or consult earlier measurements.

$\mathbf{t_{50}}$: Time, expressed in seconds, at which the measured opacity reaches 50% of the value between the maximum and minimum of the fitted curve.

$\mathbf{t_f}$: User defined time, expressed in seconds, indicating when the biomagnetic separation process is complete. It defines the final time value used for fitting the experimental curve and displaying the measurement. By default, t_f is fixed as the time when you press Force End in the measurement

Trigger: Minimum difference between two consecutives opacity measurements that makes the **Monitor** software start the data acquisition. The default value is 30.

User: Person using a **Sepmag®** system equipped with the monitoring hardware and **Monitor** software**.**

Annex: Sigmoidal curve fitting troubleshooting

If the determination coefficient r^2<0.99, the obtained fitting values (t_{50}, p, V_0, V_{max}, t_{99}) should be critically revised.

The optical changes of the suspension when it is inside the SEPMAG (i.e. when a constant magnetic force is applied) can usually be fitted to a sigmoidal curve. To test the quality of the fitting, the software provides the coefficient of determination r^2, issuing a warning if its value is less than 0.99.

A bad fitting can be caused by a bad convergence of the algorithm or because the separation curve doesn't have a sigmoidal behavior.

If you want to improve the fitting, you can:

- **Change the number of points that the algorithm doesn't take into account for the fitting**

  The firsts/lasts points of the measurements can be affected by the introduction or removal of the bottle. These points out of the expected sigmoidal behavior may mislead the algorithm. Removing the N_p firsts and lasts values will help the convergence. By default, N_p =10.

- **Filter the noise using the running average**

  Very diluted samples and/or translucid bottles may generate a low contrast, then the experimental values will be 'noisy'. Using a running average, the curve will be smoothed and will improve the determination coefficient. By default, N_{av} =1.

- **Modify the final time, t_f**

  Having too much data once the curve near its minimum value may overweight the fitting algorithm, losing detail on the initial part of it. Having not enough data after t_{50}, by contrast, will produce a loss of adjustment in the final part. We recommend a t_f between t_{99} and 3*t_{99}. For high 'p' values, t_f can be close to t_{99}. For low 'p' values, t_f can be close to $3*t_{99}$. By default, t_f is fixed as the time when you press Force End in the measurement.

In **MONITOR**, you may modify these fitting parameters at the Measuring/Retrieve Data window. At the Selected Process table, you should click the right square indicating the color of the curve and you will access to a new window where you may play with the parameters. Once 'save', they will be filed in the database Once you change and save the new parameters value, they would be applied to ALL the curves of the step of the Process and the change would be recorded in the Audit Trail.

For additional information, questions and technical support, please contact your local sepmag® representative or email

contact@sepmag.eu

For additional resources on biomagnetic separation (free eBooks, technical Posts, etc.), visit our website www.sepmag.eu