

July 2021

Rapid and Scalable Media Preparation With Powdered Media and Single-Use Lev Mixer Technology

Executive Summary

This application note presents the results of media preparations using the Flexel[®] Lev Mixer¹ technology. The method combines ready-to-use media formulations from SAFC[®], with scalable single-use mixing systems provided by Sartorius.

The performances presented in this Application Note are achieved with the superconducting drive unit that enables a levitation of the impeller encapsulated into the Flexel[®] bag for Lev Mixer. This technology is an alternative to the Flexel[®] for Magnetic Mixer² technology which is recommended by Sartorius for optimized media preparation (Flexel[®] for Magnetic Mixer is also part of Flexact[®] MP, a configurable disposable solution for media preparation).

Two examples of media preparation steps are presented: Ex-Cell[™] CD CHO Fusion and Ex-Cell[™] EBx[®] GRO-I.

The contained transfer of powdered media formulations into the Lev Mixer system enables the dissolution and dispersion of the media powders in liquid for volumes of 50 L to 1,000 L, with moderate torque. The performances of the single-use mixing system are characterized with quantitative (conductivity measurement) and qualitative (visual inspection) techniques. The proposed method and system provide seamless scale-up and consistent rapid media mixing for process development and GMP manufacturing.

Introduction

This application study presents the performances of a fully single-use mixing solution for the preparation of two different media. The mixing technology characterized in this study is Flexel® for Lev Mixer with volumes of 50 L, 200 L and 1,000 L. The levitated impeller enables a rotation speed up to 180 rpm, providing an efficient mixing of the media.

The first medium tested in this study is the Ex-Cell™ EBx® GRO-I Serum-Free for Embryonic Stem Cells. This medium is an animal-component free, serum-free dry powder formulated for the growth of EB66® cells. The EB66® cell line is proprietary to Vivalis (Saint-Herblain, France). The EB66® cell line is a fully characterized duck cell line utilized in cell-based vaccine manufacturing and for the production of recombinant viral vectors and therapeutic recombinant proteins.

The second medium tested on this study is the EX-Cell™ CD CHO Fusion. It is a chemically defined, animal-component free medium developed for the long-term growth of Chinese Hamster Ovary (CHO) cells. The absence of any large macromolecules allows for isolation and purification of secreted proteins from the cells. This medium is supplied without L-glutamine to aid in media stability, to avoid L-glutamine degradation that causes ammonia build-up and to provide an appropriate medium for the culture of CHO cells using the Glutamine Synthetase, or GS, System™. This medium does not contain hypoxanthine or thymidine to allow for its use with dihydrofolate reductase (DHFR-) gene amplification systems.

Purpose of the Application Study

The purpose of this application study is to assess the performances of the Flexel® for Lev Mixer technology to dissolve the two media. The mixing times are determined by conductivity and visual inspection of the solution in the Flexel® bag for Lev Mixer.

Materials and Methods

The list of materials and equipments used for this application is:

1. Standard Flexel® bag for Lev Mixer (50 L: FXB111567, 200 L: FXB111420, 1,000 L: FXB111569)
2. Palletank® for Lev Mixer and Magnetic Mixer (50 L: FXC110820, 200 L: FXC110821, 1,000 L: FXC113384)
3. Superconducting drive unit, 230V, EU power cord (ref. LT-DBTL-007)
4. SAFC® media:
 - Ex-Cell™ EBx® GRO-I (Product number: 24530C/44076)
 - EX-Cell™ CD CHO Fusion (Product number: 24365C/44075)
5. Sodium bicarbonate (SAFC : 90421C)
6. NaOH (1M)
7. HCl (1M)
8. Conductivity sensor: WTW InoLab Cond 740i
9. pH sensor : Knick SE 101
10. Floor scale: Sartorius IF S4 1500RR-1

¹ Lev Mixer is a trademark or registered trademark of Pall Corporation in the United States, other countries or both and this product uses Pall-patented Lev Mixer technology.

² This product uses Pall-patented Magnetic Mixer technology. All information on patents can be found at Pall.com/patents

Method Used:

1. The Flexel® bag for Lev Mixer is placed into the Palletank® with conductivity and pH sensors
 2. The bag is filled with deionised water to 80% of the final volume (water temperature: 20°C)
 3. The mixing speed is turned on and set up to the maximum speed of 180 rpm to optimize powder dispersion
 4. Media powders are added slowly through the top port to ease the powder incorporation into the water
 - Ex-Cell™ EBx® GRO-I : final concentration 19.06 g/L
 - EX-Cell™ CD CHO Fusion : final concentration 20.09 g/L
 5. When the media is dissolved, sodium bicarbonate is added:
 - Final concentration : 1.6 g/L for Ex-Cell™ EBx® GRO-I
 - Final concentration: 1.25 g/L for Ex-Cell™ CD CHO Fusion
- The powders are incorporated in the Flexel® bag for Lev Mixer using SAFC® bucket liners or the Sartorius powder transfer bag system.
6. pH is adjusted by using NaOH or HCl:
 - To 6.9 – 7.1 for the Ex-Cell™ EBx® GRO-I
 - To 7.2 - 7.4 for the EX-Cell™ CD CHO Fusion
 7. Deionised water is added to achieve the final volume
 8. The medium is filtered (step not done during the study)
 9. pH and conductivity measurements are collected to illustrate the process steps and show mixing performances:

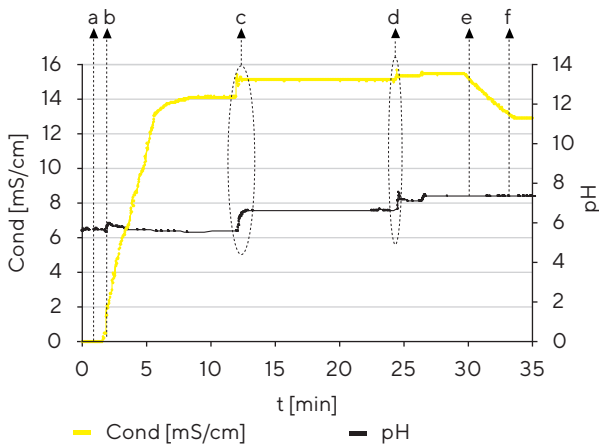


Figure 1: Media preparation process steps

- a: End of water filling (deionised water is added to 80% of the final volume)
- b: Start mixing and media powder addition
- c: Start sodium bicarbonate addition
- d: pH adjustment when needed
- e: Start final dilution (deionised water is added to the final volume)
- f: End of final dilution

10. Two mixing times are monitored from the addition of media powders:
 - 10.1 “Mixing time 1” is determined from the conductivity signal as follows:
The “mixing time 1” corresponds to the time when 95% of the final value is reached and when all next measurements stay within a 5% tolerance.

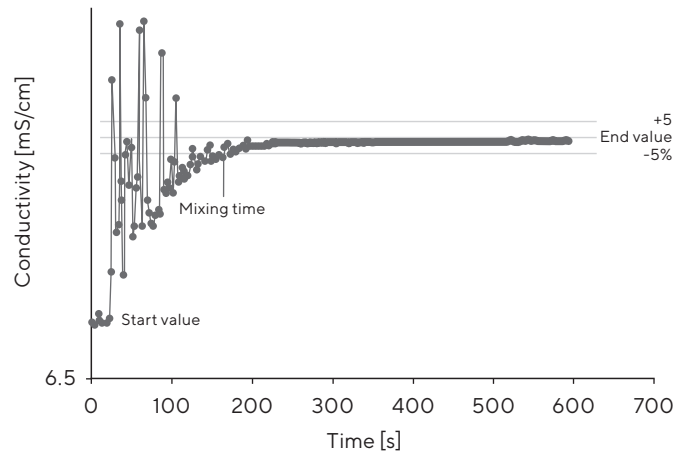


Figure 2: General principle of mixing time determination via conductivity

- 10.2 “Mixing time 2” is determined by a visual inspection. The “mixing time 2” corresponds to the time when all suspended particles are visually dissolved.



Results and Discussions

1. Mixing performances results

Ex-Cell™ CD CHO Fusion and Ex-Cell™ EBx® GRO-I media have been prepared in Flexel® bag for Lev Mixer at 50 L, 200 L and 1,000 L scales. Mixing time results are presented for each media at the different scales.

Mixing time results for Ex-Cell™ CD CHO Fusion – 50 L

For the Ex-Cell™ CD CHO Fusion preparation at 50 L, the media powder was added in less than one minute by using SAFC® bucket liners. The media powder was mixed in less than three minutes according to conductivity, and less than six minutes according to visual check. During the preparation, a pH adjustment was needed. Finally, the Ex-Cell™ CD CHO Fusion preparation at 50 L took less than 15 minutes.

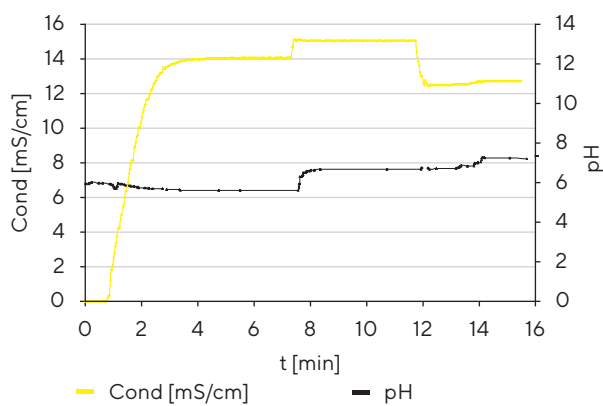


Figure 3: Ex-Cell™ CD CHO Fusion preparation in 50 L Flexel® bag for Lev Mixer

Mixing time results for Ex-Cell™ CD CHO Fusion – 1,000 L

For the Ex-Cell™ CD CHO Fusion preparation at 1,000 L, the media powder was added in less than four minutes by using SAFC® bucket liners. The media powder was mixed in less than 20 minutes according to conductivity, and in less than 30 minutes according to visual check. During the preparation, a pH adjustment was needed. Finally, the Ex-Cell™ CD CHO Fusion preparation at 1,000 L took less than 60 minutes.

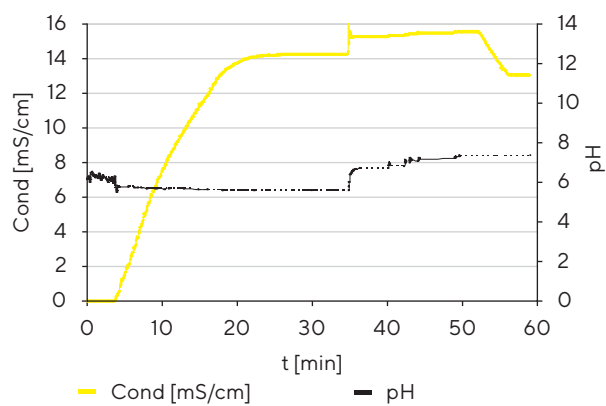


Figure 5: Ex-Cell™ CD CHO Fusion preparation in 1,000 L Flexel® bag for Lev Mixer

Mixing time results for Ex-Cell™ CD CHO Fusion – 200 L

For the Ex-Cell™ CD CHO Fusion preparation at 200 L, the media powder was added in less than three minutes. The media powder was mixed in less than four minutes according to conductivity, and in less than seven minutes according to visual check. During the preparation, a pH adjustment was needed. Finally, the Ex-Cell™ CD CHO Fusion preparation at 200 L took less than 25 minutes.

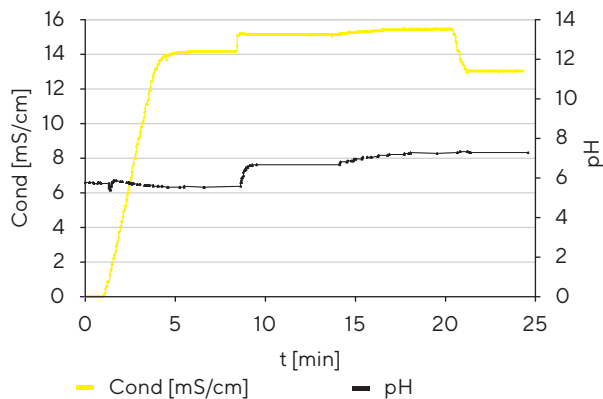


Figure 4: Ex-Cell™ CD CHO Fusion preparation in 200 L Flexel® bag for Lev Mixer

Mixing time results for Ex-Cell™ EBx® GRO-I – 50 L

For the Ex-Cell™ EBx® GRO-I preparation at 50 L, the media powder was added in less than two minutes by using SAFC® bucket liners. The media powder was mixed in less than three minutes according to conductivity, and less than eight minutes according to visual check. During the preparation, a pH adjustment was needed. Finally, the Ex-Cell™ EBx® GRO-I preparation at 50 L took less than 20 minutes.

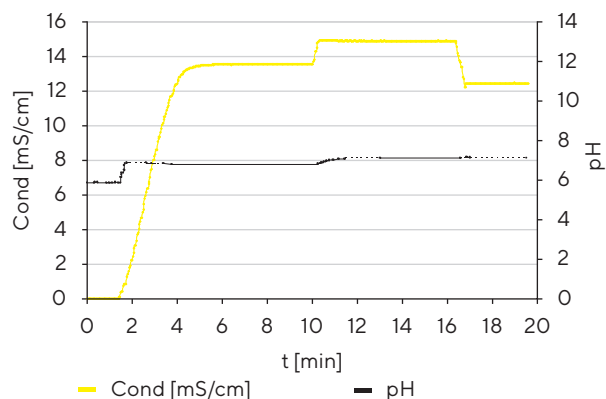


Figure 6: Ex-Cell™ EBx® GRO-I preparation in 50 L Flexel® bag for Lev Mixer

Mixing time results for Ex-Cell™ EBx® GRO-I – 200 L

For the Ex-Cell™ EBx® GRO-I preparation at 200 L, the media powder was added in less than three minutes by using the Sartorius powder transfer bag system. The media powder was mixed in less than four minutes according to conductivity, and in less than eight minutes according to visual check. During the preparation, a pH adjustment was needed. Finally, the Ex-Cell™ EBx® GRO-I preparation at 200 L took less than 20 minutes.

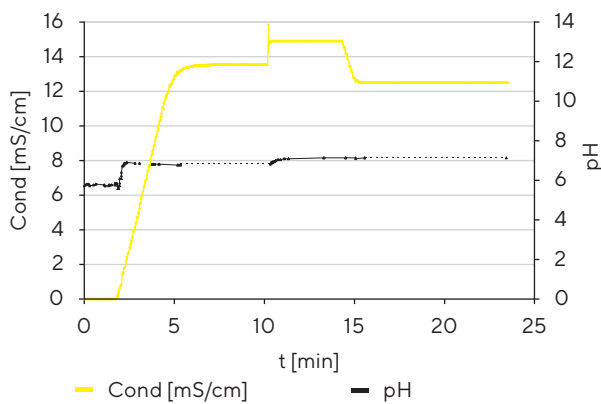


Figure 7: Ex-Cell™ EBx® GRO-I preparation in 200 L Flexel® bag for Lev Mixer

Mixing time results for Ex-Cell™ EBx® GRO-I – 1,000 L

For the Ex-Cell™ EBx® GRO-I preparation at 1,000 L, the media powder was added in less than four minutes by using SAFC® bucket liners. The media powder was mixed in less than 25 minutes according to conductivity, and in less than 35 minutes according to visual check. During the preparation, a pH adjustment was needed. Finally, the Ex-Cell™ EBx® GRO-I preparation at 1,000 L took less than 60 minutes.

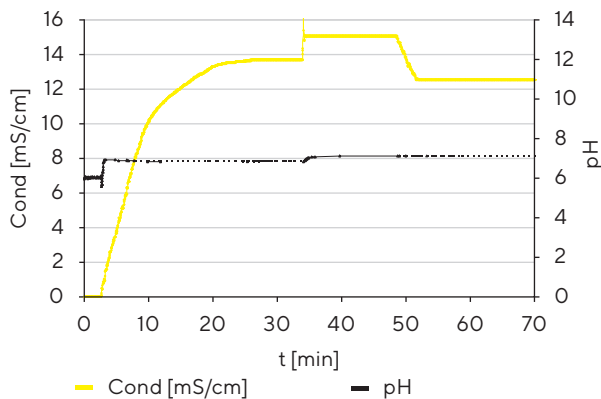


Figure 8: Ex-Cell™ EBx® GRO-I preparation in 1,000 L Flexel® bag for Lev Mixer

General comments

The mixing times reported in this study include the transfer time of SAFC® media powders into the mixing bag assembly. A rapid dissolution was observed for both Ex-Cell™ CD CHO Fusion and Ex-Cell™ EBx® GRO-I media at a rotation speed of 180 rpm.

For all volumes (50 L, 200 L and 1,000 L), the conductivity of the solutions reaches a stable value in a few minutes. However, some fine particulates can still be visually observed in the solution. The agitation at 180 rpm was maintained until the particulates became visually totally dissolved. This visual control is facilitated by the large windows of the Palletank®.

Powder bag: Ex-Cell™ CD CHO Fusion and Ex-Cell™ EBx® GRO-I media are fine powders. During both preparations, some dust appears during the media powder introduction into the Flexel® bag for Lev Mixer. The use of the powder transfer bag system docked onto the Flexel® bag is recommended to maintain a high containment and reduce the exposure of the operator to chemicals.

During Ex-Cell™ CD CHO Fusion and Ex-Cell™ EBx® GRO-I media preparation, some foam appears on the top during the media powders dissolve. The foam formation could be reduced by adding slowly the powder.

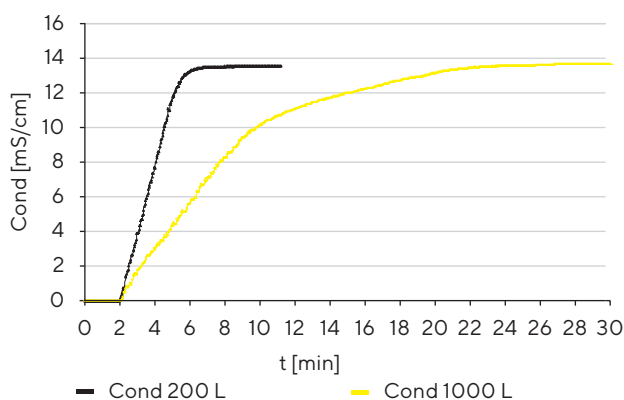
2. Mixing performance vs volume of media

The mixing performances versus the volume of media are compared only for the media powder dissolution step. In fact, the other additions (sodium bicarbonate and acid | base if needed) are almost instantaneous.

Even if the mixing time increases as expected with the volume, the media dissolution is still rapid for the 1,000 L scale experiment. The stable plateau conductivity is reached at 1,000 L in:

- Less than 25 minutes (based on conductivity measurement) for Ex-Cell™ EBx® GRO-I.
- Less than 20 minutes (based on conductivity measurement) for Ex-Cell™ CD CHO Fusion

Ex-Cell™ EBx GRO-I – 200 vs. 1,000 L



Ex-Cell™ CD CHO Fusion – 200 vs. 1,000 L

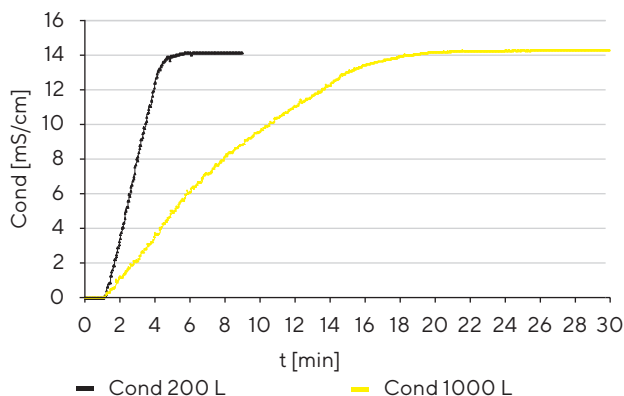


Figure 9: Media powder dissolution – comparison between 200 L and 1,000 L scales

3. Mixing performances vs media type

The mixing time reported in the table represents the time to dissolve the media powders at the different scales of Flexel® bag for Lev Mixer. These mixing times include the transfer time of the multiple SAFC® bucket liners.

Volume [L]	Control test	50	200	1,000
Media powder				
Ex-Cell™ CD CHO Fusion	Conductivity Mixing time 1	< 3 min	< 4 min	< 20 min
	Visual inspection Mixing time 2	< 6 min	< 7 min	< 30 min
Ex-Cell™ EBx GRO-I	Conductivity Mixing time 1	< 3 min	< 4 min	< 25 min
	Visual inspection Mixing time 2	< 8 min	< 8 min	< 35 min

Figure 10: Overview on mixing times regarding media powder dissolution

The process time for both media preparations including water filling, media powder addition, sodium bicarbonate addition, acid and base adjustment and final dilution takes:

- Less than 25 minutes for 50 L and 200 L scale
- Less than 60 minutes for 1,000 L scale.

Flexel® bag for Magnetic Mixer is the recommended technology to optimize the time for media powders dissolution. Performances with Magnetic Mixer are available in the application note “Rapid and scalable media preparation with powdered media and Sartorius single-use magnetic mixing systems”.

Conclusion


- Large-volume media solutions are quick and easy to prepare using the combination of ready-to-use media formulations and the Flexel® for Lev Mixer technology.
- Sartorius recommends to use the Flexel® for Magnetic Mixer technology for optimized dissolution time of the media powders (performances are detailed in the Sartorius Application Note #16).
- The contained processing conditions with the closed powder transfer bag system docked onto the sterile Flexel® bag for Lev Mixer are recommended to maintain low bioburden and to reduce to the minimum exposure of the operator to chemicals.
- The platform provides a single-use scalable media preparation capability with a range of Flexel® bags including volumes of 50 L, 100 L, 200 L, 400 L, 650 L and 1,000 L.

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Publication No.: SL-1085-e | Order No.: 85032-540-21 | Status: 10 | 07 | 2021