



## Application Note

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### Flexsafe® Pro Mixer

The Fast, Flexible and Intelligent Solution for Media and Feed Preparation

Delphine Audubey<sup>1\*</sup>, Adiba Ahmad<sup>2\*</sup>, Anilkumar Paramathma<sup>3</sup>

1. Sartorius Stedim FMT S.A.S., ZI des Paluds, Avenue de Jouques – CS 91051, 13781 Aubagne Cedex

2. Sartorius Stedim North America Inc., 545 Johnson Avenue, Bohemia, NY 11716

3. Sartorius Stedim India Pvt. Ltd., #69/2-69/3, NH 48, Jakkasandra, Nelamangala Tq, 562 123 Bangalore, India

\*Correspondence

E-Mail: [delphine.audubey@sartorius-stedim.com](mailto:delphine.audubey@sartorius-stedim.com)

### Abstract

Flexsafe® Pro Mixer is a unique single-use technology fitting all mixing applications from buffer and media preparations, downstream processes to final formulation. Flexsafe® Pro Mixer ergonomic design enables intuitive, modular and agile use to achieve fast installation and mixing operations. Additionally, the Flexsafe® film offers high standard quality attributes such as Biocompatibility, Integrity and Supply network.

This application study gives performance data of the Pro Mixer for the dissolution of CHO media in 50 L, 200 L and 1000 L and 50X Soy hydrolysate in 50 L. These solutions are used in media and feed preparation steps of the biomanufacturing process.

The powders used to manufacture these solutions are floating and form clumps making them difficult to hydrate. The Soy hydrolysate is prepared in 50X, high concentrated solution to allow use in larger volume media preparation using the appropriate dilution. The performance of the single-use mixing system is assessed using conductivity for quantitative measurement and visual inspection for qualitative measurement.

Flexsafe® Pro Mixer is able to mix CHO media in less than 5 minutes for the 50 L and 200 L, less than 15 minutes for the 1000 L and the 50X Soy hydrolysate in less than 20 minutes in 50 L.

## Introduction

The purpose of this application study is to assess the performance of the single-use Pro Mixer used in media and feed preparations.

The CHO media used is a chemically defined, non-animal origin, protein-free cell culture medium developed to deliver high levels of cell proliferation, protein production, and cell viability for the bioproduction of therapeutic recombinant proteins and monoclonal antibodies in Chinese Hamster Ovary (CHO) cells.

The Soy hydrolysate used is an ultra-filtered animal free hydrolysate, based on soy protein. Soy hydrolysate is used to support the growth and expression of product in biopharmaceutical cell cultures and fermentations. It is prepared as a 50X concentrated solution in order to fulfill needs for large scale bioproduction which constitute a worse case scenario in terms of dissolution.

These powders can be difficult to hydrate as they tend to float on the fluid surface and form clumps.

The procedure for media and feed preparation includes the incorporation of the powders in the bag partially filled with deionized water before final dilution. In this study, the bags were respectively filled to 80% of the nominal volume prior to CHO media powder addition and 70% of the nominal volume prior to Soy hydrolysate powder addition. The final step of the process consists of the addition of supplements like lipids for example, then pH adjustment and water fill to nominal volume (not described in this note). The final media and feed concentrations are described in "Materials" section.

The mixing technology selected for this application is Flexsafe® for Pro Mixer with volumes of 50 L, 200 L, and 1,000 L for the media and 50L for the concentrated feed. The magnetic coupling of the impeller with the Pro Mixer Drive Unit enables a rotation speed up to 750 rpm, providing a powerful mixing of the media and feed powders. In order to work under customer conditions, the speed has been adjusted depending on the final volume to avoid foaming effects or splashing (see "Method" section).

The mixing times are determined by conductivity measurement of the solution in the Flexsafe® bag for Pro Mixer using the single-use conductivity probe from the bags. These data are confirmed by visual inspection using several cameras including a submersible camera to perform comprehensive and thorough checks at the surface, throughout the entire volume and in the 4 bottom corners.



## Materials and Methods

### Materials

#### Consumable

- Standard Flexsafe® Bags for Pro Mixer (50 L, 200 L and 1000L) including single-use conductivity sensors
- Powder bags (15 L and 30 L)
- Powders:
  - PowerCHO at 29.14 g/L final concentration
  - Proyield Soy at 250 g/L final concentration

As mixing is performed at 80% and 70% of the nominal volume, the concentration during the mixing phase is higher.

- Deionized water

#### Equipment

- Palletank for Mixing equipped with Powder bag holder
- Pro Mixer drive unit
- 3 different type of camera: video camera, still camera and submersible camera type GoPro

### Method

1. The CHO media is prepared in standard Flexsafe® Bags for Pro Mixer filled with deionized water to 80% of the final volume. The 50X Soy hydrolysate is prepared in standard Flexsafe® Bags for Pro Mixer filled with deionized water to 70% of the final volume.

2. Impeller speed is set based on the nominal test volume to avoid foaming effects or splashing:

- 325 rpm for 50L
- 475 rpm for 200L
- 725 rpm for 1000L

3. The powders are incorporated in the Flexsafe® Bags for Pro Mixer using 15 L or 30 L powder transfer bag for a contained transfer to the mixing bag assembly.

4. Two mixing times are monitored, these mixing times includes powders' addition into the Flexsafe® Bags for Pro Mixer: "Mixing time 1" is determined from the conductivity signal.

The "mixing time 1" corresponds to the time when 99% of the final conductivity value is reached and when all next measurements stay within a 1% tolerance for at least 5 minutes.

"Mixing time 2" is determined by a visual inspection.

The "mixing time 2" corresponds to the time when all suspended particles are visually dissolved and no visible solids remain floating on the fluid surface. Several external and submersible cameras are recording the experiment allowing, among others, to perform comprehensive checks on the fluid volume and surface.

Total mixing time corresponds to the highest value among mixing 1 and mixing 2.

## Results

### 1. 50 L CHO media mixed in less than 5 minutes

The CHO media is dissolving rapidly, in less than 1 minute which is confirmed by visual inspection.

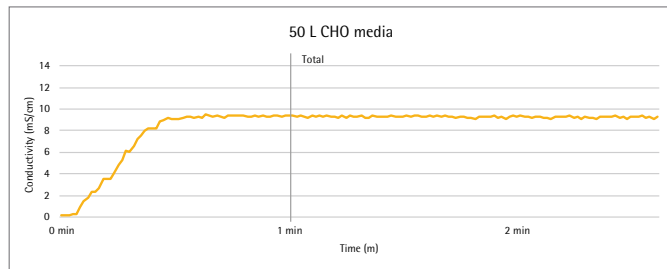


Fig. 1: CHO media preparation in 50 L Flexsafe® Bag for Pro Mixer

### 2. 200 L CHO media mixed in less than 5 minutes

The CHO media is dissolving rapidly, in less than 3 minutes which is confirmed by visual inspection.

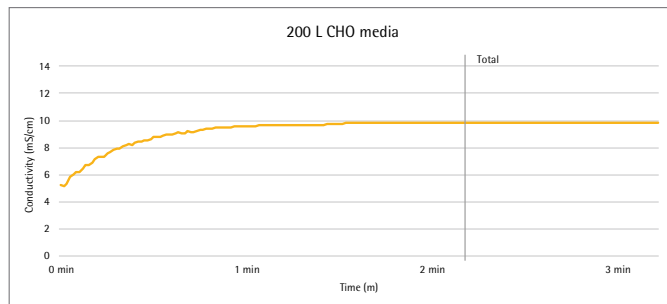


Fig. 2: CHO media preparation in 200 L Flexsafe® Bag for Pro Mixer

### 3. 1000 L CHO media mixed in less than 15 minutes

The conductivity is stabilizing after 10 minutes and full dissolution is confirmed by visual inspection in less than 15 minutes.

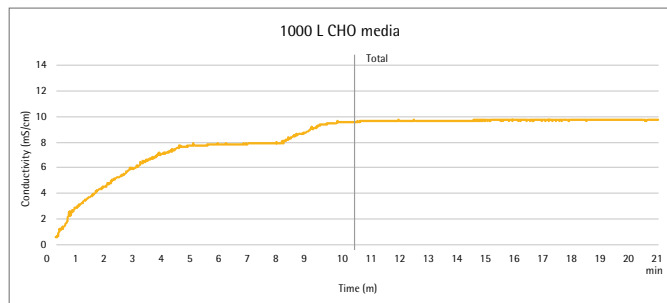


Fig. 3: CHO media preparation in 1,000 L Flexsafe® Bag for Pro Mixer



Fig. 4: CHO media floating on top after powder addition



Fig. 5: Clumps at the surface during mixing



Fig. 6: No remaining clumps at the end of CHO media dissolution

### 4. 50 L 50X Soy hydrolysate mixed in less than 20 minutes

Soy hydrolysate is prepared in high 50x concentration which represents a worse case. This solution is foaming when agitated and even with the 325 rpm set-up for this volume, foaming was observed during the test. The small conductivity variations seen on the graph confirms the presence of bubbles around the conductivity probes due to foaming.

Despite the high concentration of this feed solution, the system was able to achieve a full mixing in less than 20 minutes with a stabilization of the conductivity in less than 10 minutes.

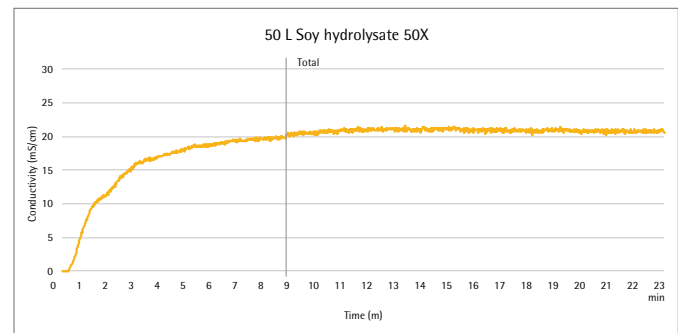


Fig. 7: 50X Soy hydrolysate preparation in 50 L Flexsafe® Bag for Pro Mixer

## 5. Summary of the Flexsafe® Pro Mixer performance

Powder	Dissolution Characteristic	Mixing Time Measurement	Mixing Time per Volume		
			50 L	200 L	1000 L
CHO Media	Floating and forming clumps	Conductivity	< 5 min	< 5 min	< 10 min
		Visual inspection	< 5 min	< 5 min	< 15 min
50x Soy hydrolysate	Worse case: high concentration and floating clumps	Conductivity	< 10 min	-	-
		Visual inspection	< 20 min	-	-

Table 1: Summary of mixing times obtained with Pro Mixer System

## Discussion

A rapid dissolution of the powders was observed in each case tested. The mixing time was found to be below 5 minutes for the CHO media in 50 L and 200 L, below 15 minutes for the 1000L and a maximum mixing time below 20 minutes for the worse case, 50X Soy hydrolysate in 50 L.

Despite the clumps forming at the surface, the Pro Mixer vortex was able to attract down the powders for an efficient mixing.



Fig. 8: Vortex at 475 rpm in a 200 L Flexsafe® Bag for Pro Mixer

The fluid dispersion in the tank has been also studied with CFD analysis, confirming the existence of ascending and descending flows without any stagnant areas as well as the formation of a recirculation loop above the impeller for an efficient mixing process. A detailed technical note is available on this topic: "Flexsafe® Pro Mixer Computational Fluid Dynamic studies".

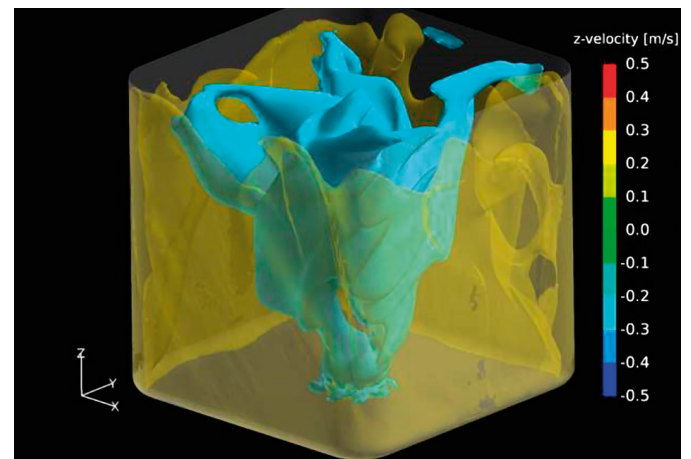


Fig. 9: 3D view – Simulation of vertical velocity in 1.000 L Pro Mixer at 750 rpm

The mixing times presented in this application note do not include the time it takes for: equipment set up, water filling to 70% or 80%, as well as supplement additions and water filling up to 100% to reach final volume and concentration of a given solution. Thanks to its ergonomic design, the complete set-up and bag installation of the Flexsafe® Pro Mixer is fast, 5 to 10 minutes for a 1,000 L bag.

## Conclusion

Flexsafe® Pro Mixer is a unique single-use technology platform fitting all mixing applications from buffer and media preparations, downstream processes to final formulation in 50 L, 100 L, 200 L, 400 L, 650 L and 1,000 L volumes.

This application demonstrates the efficiency of the Flexsafe® Pro Mixer to facilitate floating powders inclusion into the liquid and dissolution even in worse case conditions such as high concentration, making the media and feed preparation step quick and easy to perform.

Flexsafe® Pro Mixer allows for quick set-up, efficient mixing and fast changeover to save time at all the mixing steps during biomanufacturing.

FlexAct® Media Preparation unit with Flexsafe® Pro Mixer system provides a fully qualified and automated single-use solution for a more robust, productive and efficient process.

Sartorius Stedim Biotech can also provide standard and custom cell culture media and feed for proteins, vaccines and regenerative medicine applications.



# Sales and Service Contacts

For further contacts, visit [www.sartorius-stedim.com](http://www.sartorius-stedim.com)

## Europe

### Germany

Sartorius Stedim Biotech GmbH  
August-Spindler-Strasse 11  
37079 Goettingen

Phone +49.551.308.0

Sartorius Stedim Systems GmbH  
Robert-Bosch-Strasse 5-7  
34302 Guxhagen

Phone +49.5665.407.0

### France

Sartorius Stedim FMT S.A.S.  
ZI des Paluds  
Avenue de Jouques - CS 91051  
13781 Aubagne Cedex

Phone +33.442.845600

Sartorius Stedim France SAS  
ZI des Paluds  
Avenue de Jouques - CS 71058  
13781 Aubagne Cedex

Phone +33.442.845600

### Austria

Sartorius Stedim Austria GmbH  
Modectcenterstrasse 22  
1030 Vienna

Phone +43.1.7965763.18

### Belgium

Sartorius Stedim Belgium N.V.  
Rue Colonel Bourg 105  
1030 Bruxelles

Phone +32.2.756.06.80

### Hungary

Sartorius Stedim Hungária Kft.  
Kagyló u. 5  
2092 Budakeszi

Phone +36.23.457.227

### Italy

Sartorius Stedim Italy S.r.l.  
Via dell'Antella, 76/A  
50012 Antella-Bagno a Ripoli (FI)

Phone +39.055.63.40.41

### Netherlands

Sartorius Stedim Netherlands B.V.

Phone +31.30.60.25.080

[filtratie.nederland@sartorius-stedim.com](mailto:filtratie.nederland@sartorius-stedim.com)

### Poland

Sartorius Stedim Poland Sp. z o.o.  
ul. Wrzesinska 70  
62-025 Kostrzyn

Phone +48.61.647.38.40

### Russian Federation

LLC "Sartorius Stedim RUS"  
Vasilyevsky Island  
5<sup>th</sup> line 70, Lit. A  
199178 St. Petersburg

Phone +7.812.327.53.27

### Spain

Sartorius Stedim Spain, S.A.U.  
Avda. de la Industria, 32  
Edificio PAYMA  
28108 Alcobendas (Madrid)

Phone +34.913.586.098

### Switzerland

Sartorius Stedim Switzerland AG  
Ringstrasse 24 a  
8317 Tagelswangen

Phone +41.52.354.36.36

### U.K.

Sartorius Stedim UK Ltd.  
Longmead Business Centre  
Blenheim Road, Epsom  
Surrey KT19 9 QQ

Phone +44.1372.737159

### Ukraine

LLC "Sartorius Stedim RUS"  
Post Box 440 "B"  
01001 Kiev, Ukraine

Phone +380.44.411.4918

## Africa

### Republic of South Africa

Sartorius South Africa (Pty) Ltd  
Unit 4, Alphen Square South  
853 16th Road  
Midrand 1685, RSA

Phone +27.11.315.5444

## Americas

### USA

Sartorius Stedim North America Inc.  
565 Johnson Avenue  
Bohemia, NY 11716

Toll-Free +1.800.368.7178

### Argentina

Sartorius Argentina S.A.  
Int. A. Ávalos 4251  
B1605ECS Munro  
Buenos Aires

Phone +54.11.4721.0505

### Brazil

Sartorius do Brasil Ltda  
Avenida Senador Vergueiro 2962  
São Bernardo do Campo  
CEP 09600-000 - SP- Brasil

Phone +55.11.4362.8900

### Mexico

Sartorius de México, S.A. de C.V.  
Libramiento Norte de Tepotzotlan s/n,  
Colonia Barrio Tlacateco,  
Municipio de Tepotzotlan,  
Estado de México,  
C.P. 54605

Phone +52.55.5562.1102

[leadsmex@sartorius.com](mailto:leadsmex@sartorius.com)

## Asia | Pacific

### Australia

Sartorius Stedim Australia Pty. Ltd.  
Unit 5, 7-11 Rodeo Drive  
Dandenong South Vic 3175

Phone +61.3.8762.1800

### China

Sartorius Stedim (Shanghai)  
Trading Co., Ltd.  
3rd Floor, North Wing, Tower 1  
No. 4560 Jinke Road  
Zhangjiang Hi-Tech Park  
Pudong District  
Shanghai 201210, P.R. China

Phone +86.21.6878.2300

Sartorius Stedim (Shanghai)  
Trading Co., Ltd.  
Beijing Branch Office  
No. 33 Yu'an Road  
Airport Industrial Park Zone B  
Shunyi District, Beijing 101300

Phone +86.10.8042.6501

Sartorius Stedim (Shanghai)  
Trading Co., Ltd.  
Guangzhou Branch Office  
Room 1105  
Xing Guang Ying Jing Building  
No. 119, Shui Yin Road  
Yue Xiu District, Guangzhou 510075

Phone +86.20.3836.4193

### India

Sartorius Stedim India Pvt. Ltd.  
#69/2-69/3, NH 48, Jakkasandra  
Nelamangala Tq  
562 123 Bangalore, India

Phone +91.80.4350.5250

### Japan

Sartorius Stedim Japan K.K.  
4th Fl., Daiwa Shinagawa North Bldg.  
8-11, Kita-Shinagawa 1-chome  
Shinagawa-ku, Tokyo, 140-0001 Japan

Phone +81.3.4331.4300

### Malaysia

Sartorius Stedim Malaysia Sdn. Bhd.  
Lot L3-E-3B, Enterprise 4  
Technology Park Malaysia  
Bukit Jalil  
57000 Kuala Lumpur, Malaysia

Phone +60.3.8996.0622

### Singapore

Sartorius Stedim Singapore Pte. Ltd.  
10 Science Park Rd  
The Alpha #02-13/14  
Singapore Science Park II  
Singapore 117684

Phone +65.6872.3966

### South Korea

Sartorius Korea Biotech Co., Ltd.  
8th Floor, Solid Space B/D,  
PanGyoYeok-Ro 220, BunDang-Gu  
SeongNam-Si, GyeongGi-Do, 463-400

Phone +82.31.622.5700



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