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Application Note

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Flexsafe® Pro Mixer

The Fast, Flexible and Intelligent Solution for Large Volume Buffer Preparation

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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 presents performance data of the Pro Mixer from 1,500 L to 3,000 L using Tris buffer. Tris buffer is commonly used in downstream steps of the biomanufacturing processes.

The performances of the single-use mixing system are assessed using quantitative (conductivity and pH measurement) and qualitative (visual inspection) measurements.

Flexsafe® Pro Mixer is able to mix 1500 L of Tris buffer in less than 5 min and 3000 L in less than 15 minutes.

Introduction

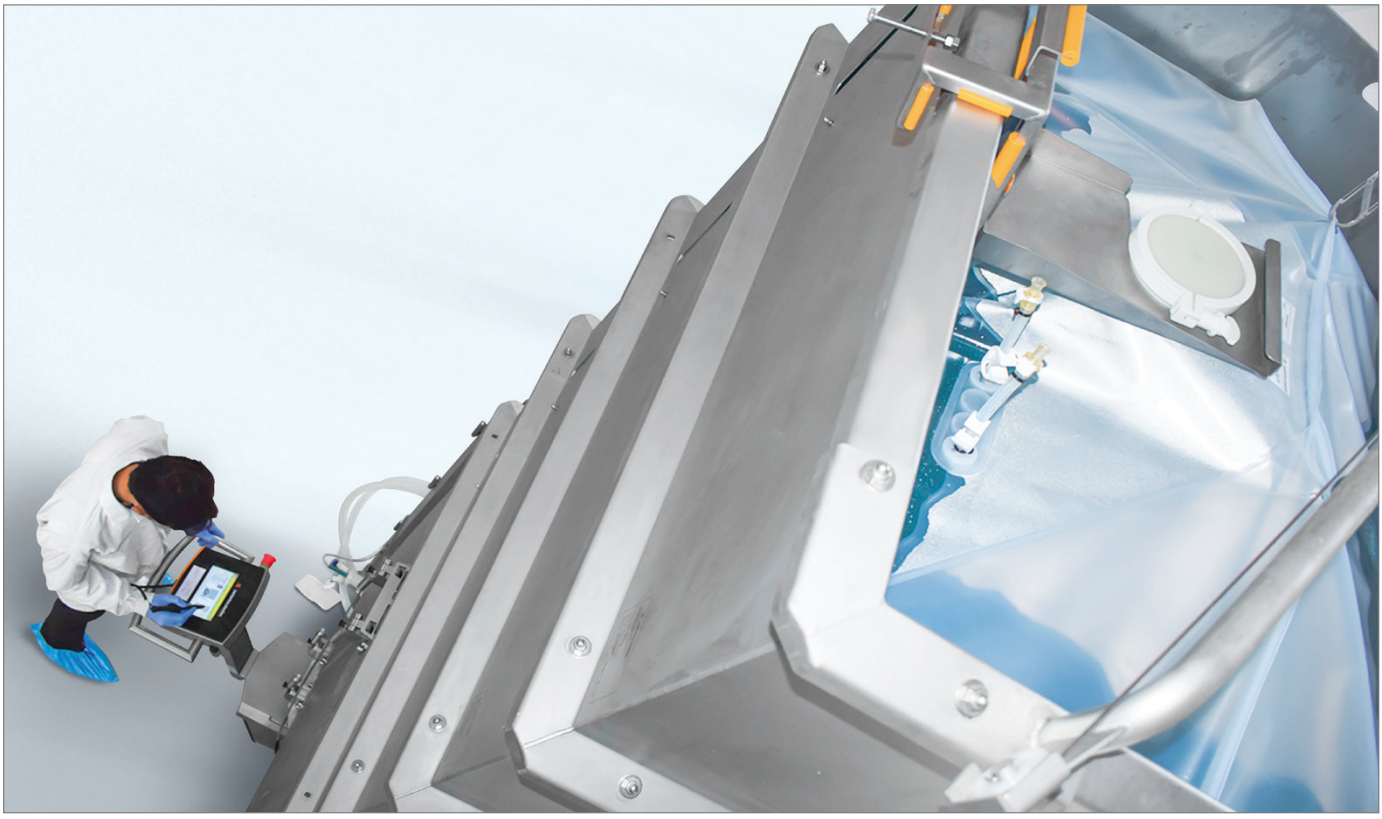
The purpose of this application study is to assess the performance of the single-use Pro Mixer at large volume. To this end, Tris buffer is prepared according to table 2. Tris buffer is commonly used in downstream steps like pH equilibration, Tangential Flow Filtration and final formulation.

The procedure 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 powder addition. The final step of the process consists in the addition of water to achieve the expected buffer concentrations. The final concentrations of salts are listed in table 2.

The mixing technology selected for this application is Flexsafe® for Pro Mixer with volumes of 1,500 L, 2,000 L, 2,500 L and 3,000 L.

The magnetic coupling of the impeller with the Pro Mixer Drive Unit enables a rotation speed of 750 rpm, providing a powerful mixing of the buffer salts at large volume.

The mixing times are determined by conductivity and pH when relevant of the solution in the Flexsafe® bag for Pro Mixer using the single-use probes from the bags. These data are confirmed by visual inspection using several cameras including a submersible camera to perform comprehensive and thorough checks on the 4 bottom corners.



Materials and Methods

Materials

Consumable

- Standard Flexsafe® Bags for Pro Mixer (1,500 L, 2,000 L, 2,500 L and 3,000 L) including single-use pH and conductivity sensors
- Powder bags (15 L and 30 L)
- Powders (listed in table 2).
- Deionized water

Buffer	Material	Final Concentration (g/L)	Amount of powder added (kg) for a 3000 L preparation
	Sodium Chloride	58.44	175.32
Tris	TRIS Base	6.06	18.18
	TRIS HCL	3.15	9.45

Table 1: Tris buffer recipe given for 3000 L final volume. The final concentration is the same for all the volume tested so the amount of powder added for other volumes can be easily calculated accordingly. As mixing is performed at 80% of nominal volume, the concentration during the mixing phase is even higher.

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 buffer is prepared in standard 1,500 L, 2,000 L, 2,500 L and 3,000 L Flexsafe® Bags for Pro Mixer filled with deionized water to 80% of the final volume.

2. Impeller speed is set at 750 rpm.

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 buffer powders' addition:
"Mixing time 1" is determined from the conductivity and pH signals.
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 and when 95% of the final pH value is reached and when all next measurements stay within a 5% 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. Several external and submersible cameras are recording the experiment allowing, among others, to perform comprehensive checks on the 4 bottom corners.

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

Mixing trial performed at 3000L scale

Results

1. 1,500 L Tris buffer mixed in less than 5 minutes

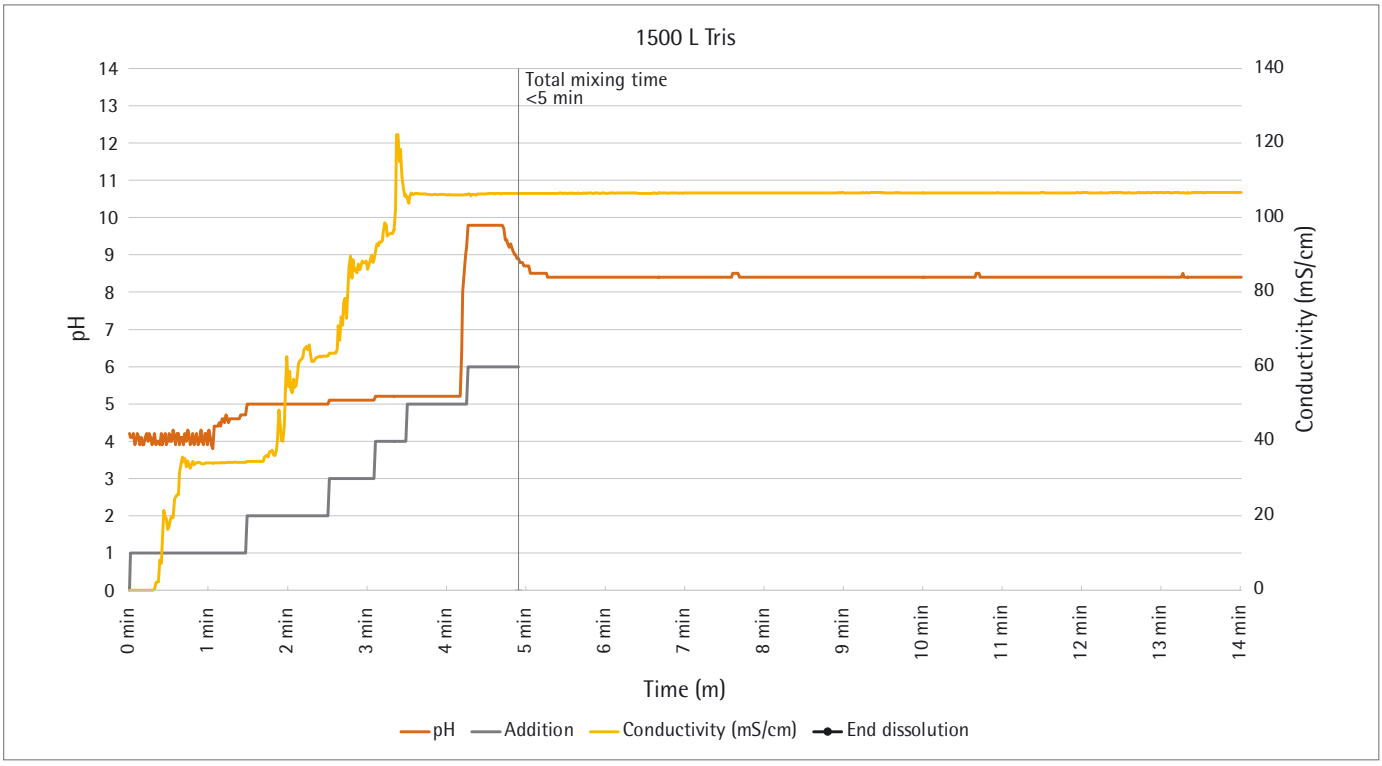
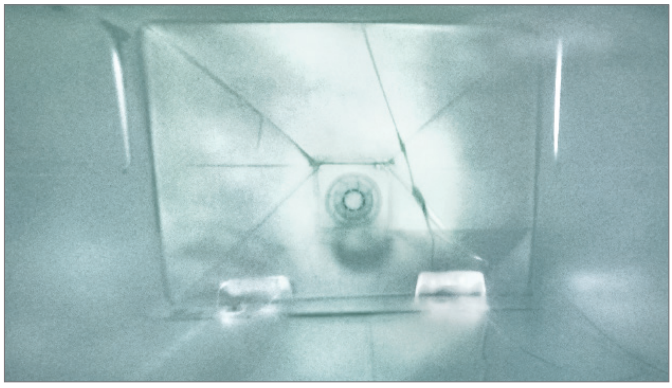


Fig. 1: Tris buffer preparation in 1,500 L Flexsafe® Bag for Pro Mixer

After powder addition



After complete dissolution

2. 2,000 L Tris buffer mixed in less than 10 minutes

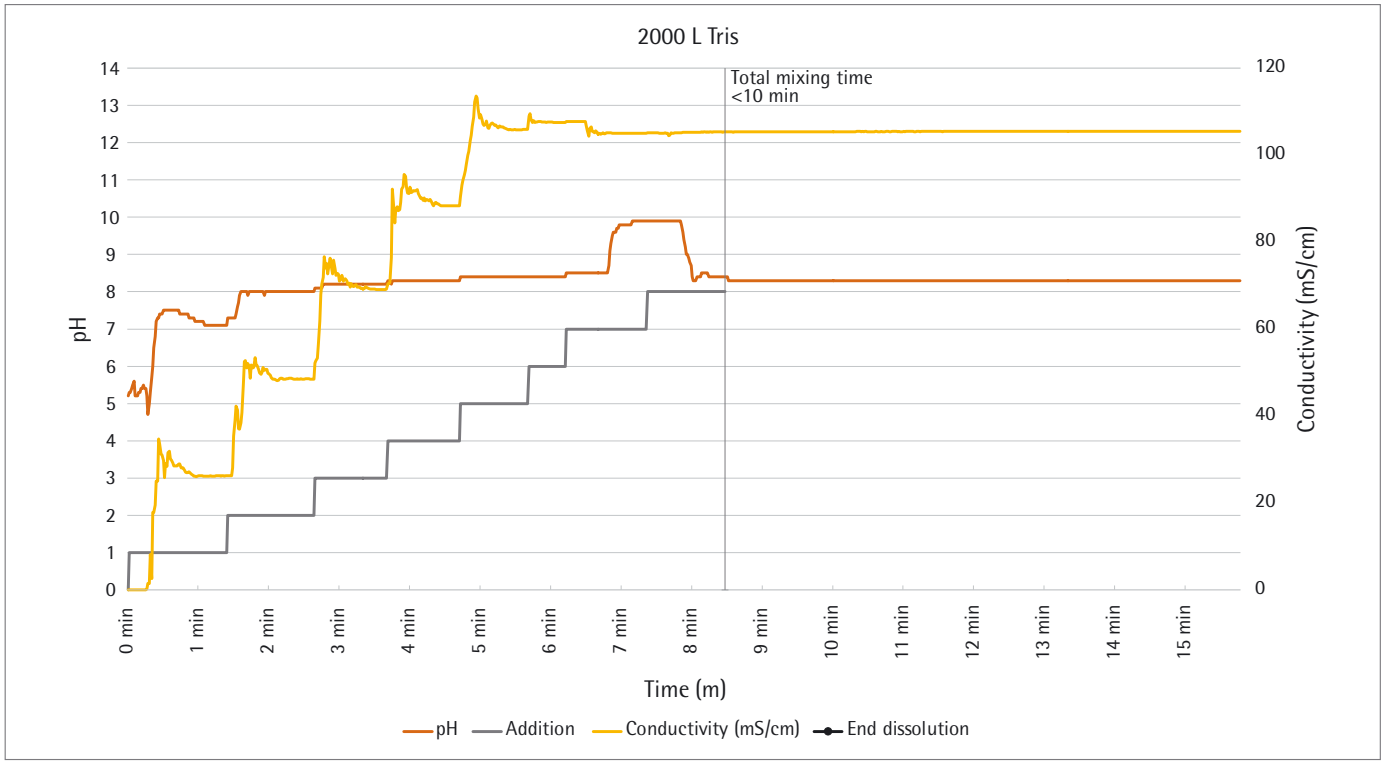


Fig. 2: Tris buffer preparation in 2,000 L Flexsafe® Bag for Pro Mixer

3. 2,500 L Tris buffer mixed in less than 15 minutes

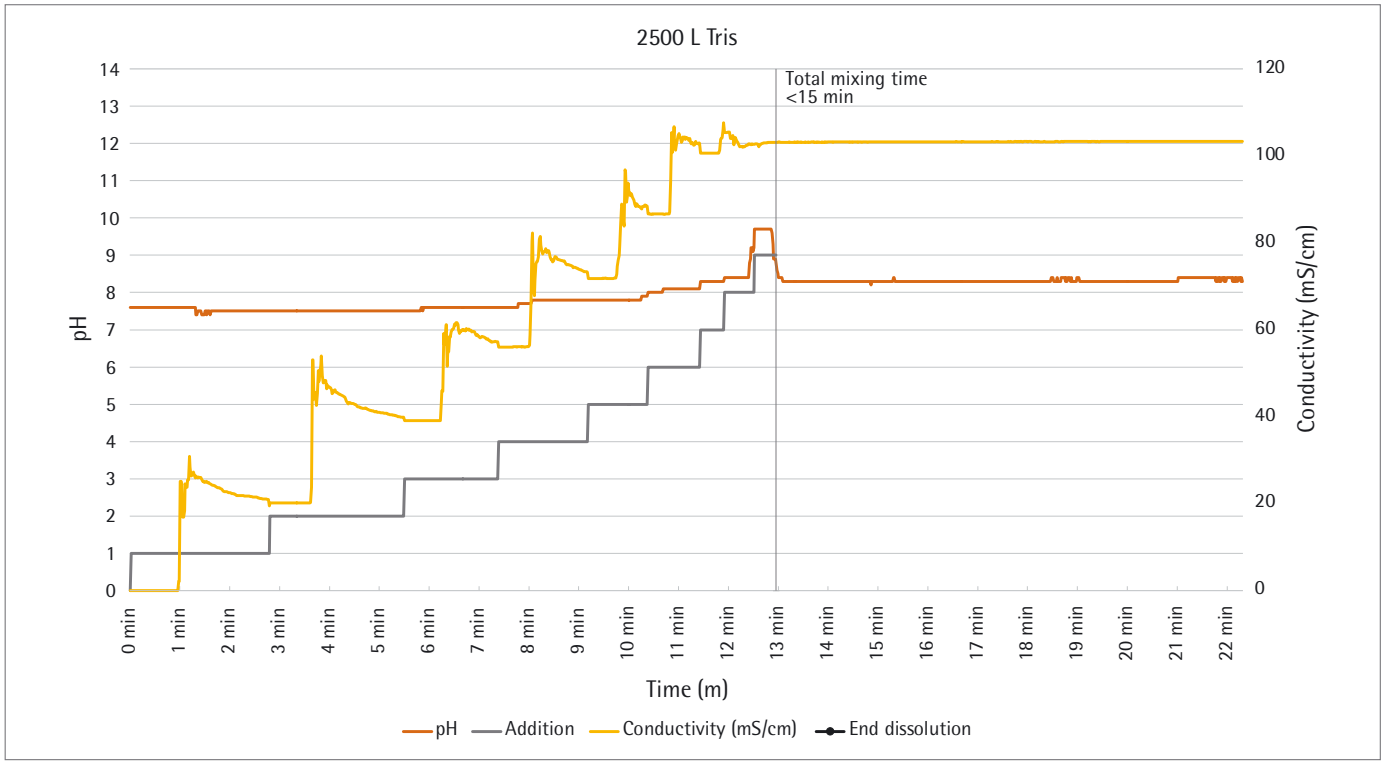


Fig. 3: Tris buffer preparation in 2,500 L Flexsafe® Bag for Pro Mixer

4. 3,000 L Tris buffer mixed with more than 200 kg of powder in less than 15 minutes

Large volume buffer applications usually require addition of large amount of powder. In the first step of the 3000 L Tris buffer preparation, 10 powder bags for a total of more than 200 kg of powder amounts were added into 2400 L of water. The Flexsafe® for Pro Mixer was able to dissolve and mix this solution in less than 15 minutes.

On the graph, we can observe pH variations due to the addition of Tris base and Tris HCL.

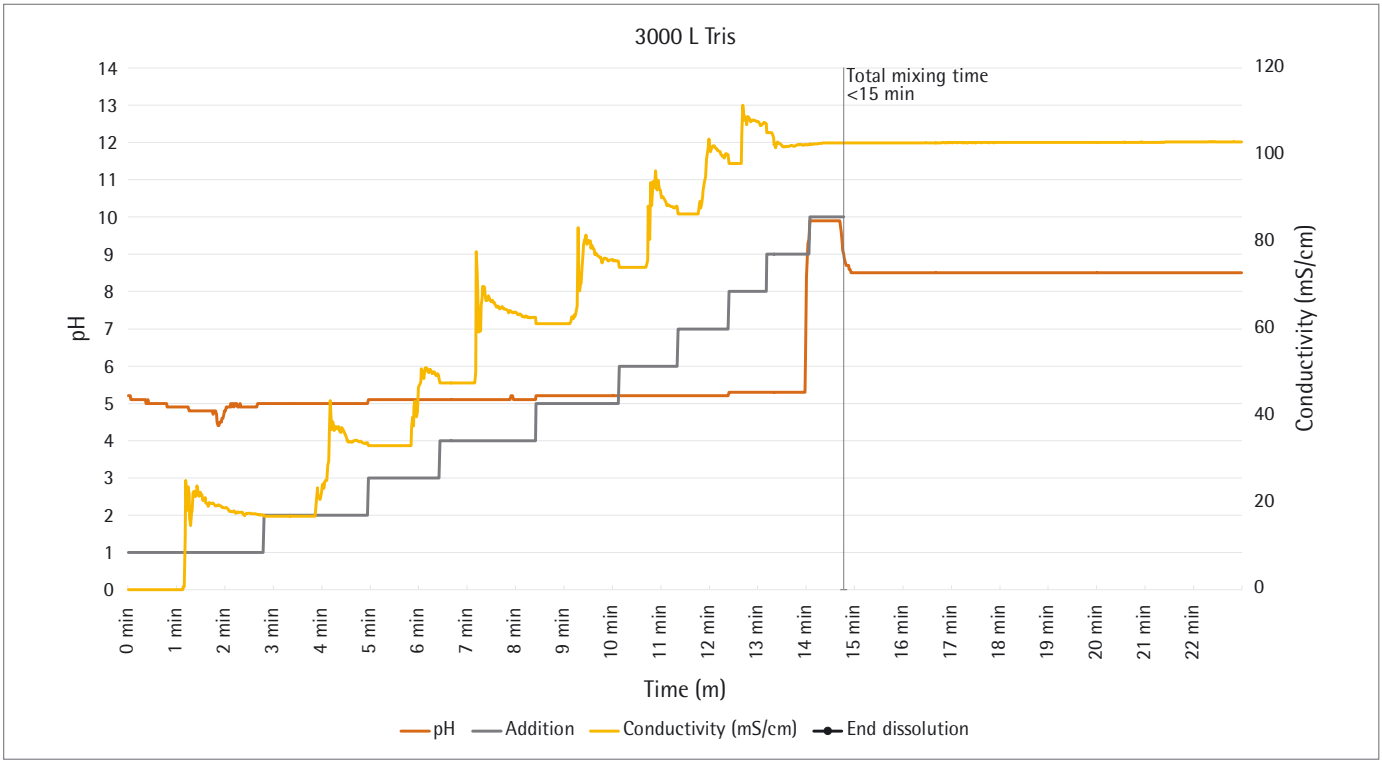


Fig. 4: Tris buffer preparation in 3,000 L Flexsafe® Bag for Pro Mixer

5. Large volumes of Tris buffer mixed in less than 15 minutes from 1,500 L to 3,000 L

Buffer	Typical Application	Dissolution Characteristic	Mixing Time Measurement	Mixing Time per Volume 1500 L	2000 L	2500 L	3000 L
Tris buffer	pH equilibration, Tangential Flow Filtration and final formulation	Common buffer used to test large volume performances up to 3000 L	Conductivity & pH	<5 min	<10 min	<15 min	<15 min
			Visual inspection	<5 min	<10 min	<10 min	<10 min

Table 2: Summary of mixing time obtained to prepare Tris buffer with large volume Pro Mixer System.

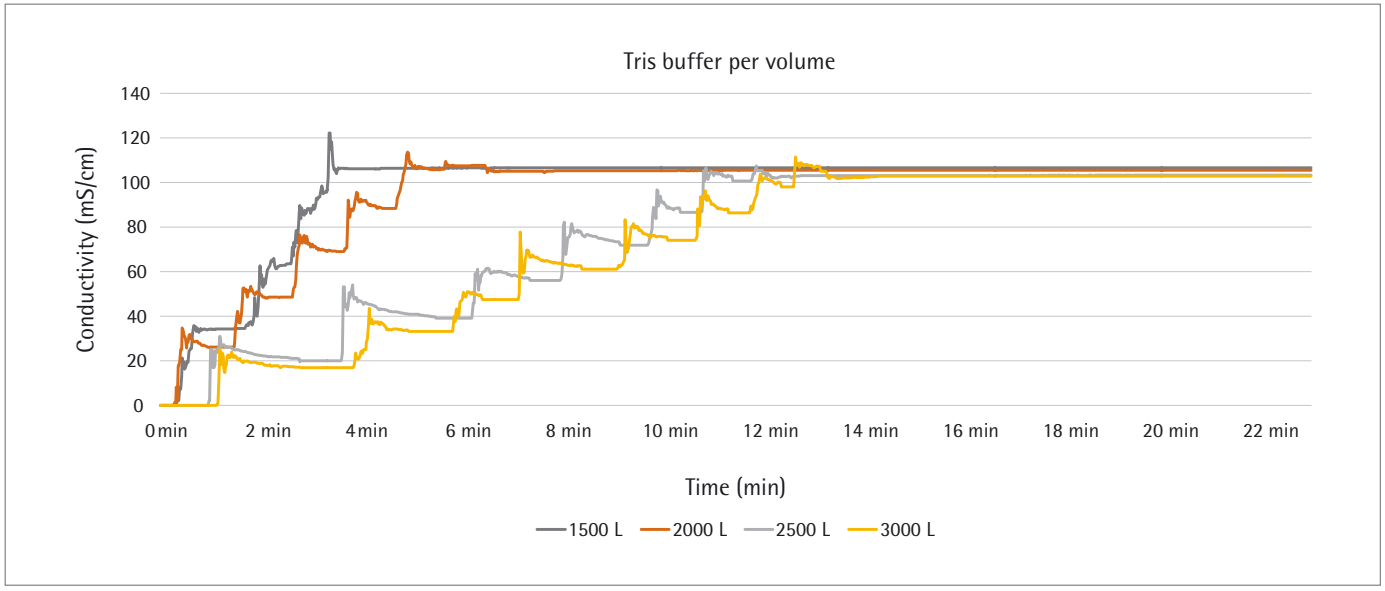


Fig. 5: Tris buffer preparation in 1,500 L, 2000 L, 2,500 L, and 3,000 L Flexsafe® Bag for Pro Mixer

Discussion

A rapid dissolution of the powder salts was observed for each volume. With a maximum mixing time below 15 min for the larger volumes (2500 L and 3000 L) and a mixing time below 5 minutes for the 1500 L, the Pro Mixer demonstrated very good efficiency for large volume buffer preparation applications.

The mixing times reported in this study include the transfer time of multiple Sartorius Stedim Biotech powder transfer bags into the mixing bag assembly. Each powder bag was filled with a maximum of 23 kg of powder. This is compliant with the ergonomic regulations that ensure proper operator safety. The overall mixing time is thus increased as the number of powder bags added during the process increases with the bag volume. For example, 10 bags have been added to prepare the 3000 L Tris buffer.

Even though the solution was clear in less than 10 minutes for the 2500 L and the 3000 L, mixing was maintained until the conductivity data reached the 99% target range (mixing time 1), in less than 15 minutes.

The mixing times presented in this application note do not include the time it takes for: equipment set up, water filling to 80%, as well as water filling from 80% 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, less than 10 minutes at 3000 L.

Conclusion

Flexsafe® Pro Mixer is a unique single-use technology platform fitting large volume buffer mixing applications up to 3000 L.

This application demonstrates the efficiency of the Flexsafe® Pro Mixer to dissolve buffer powders at large scale, making the buffer preparation step quick and easy to perform up to 3000 L.

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

FlexAct® Buffer 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 powder buffers for any downstream applications.

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