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Application Note

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Buffer Dissolution With the Flexsafe® Pro Mixer at 5, 10, and 20 L Scales

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Abstract

The Flexsafe® Pro Mixer is a state-of-the-art solution designed for efficient buffer preparation in biopharmaceutical applications. This study evaluates its performance across three buffer types—Tris, DPBS, and sodium citrate—using 5, 10, and 20 L mixer bags. The results demonstrate rapid and consistent dissolution, with conductivity reaching 99% of its final value swiftly across all tested volumes. Visual inspections confirmed complete dissolution within minutes without foaming or splashing. These findings highlight the mixer's adaptability and reliability, making it a valuable solution for processes requiring precise buffer preparation, such as protein purification, cell culture, and vaccine formulation.

For further information, visit
sartorius.com/en/products/fluid-management/mixing/flexsafe-pro-mixer

Introduction

The preparation of buffers is a critical step in various biopharmaceutical processes, including protein purification, cell culture, and vaccine formulation. The Flexsafe® Pro Mixer offers an innovative solution for efficient and reliable buffer preparation, accommodating different volumes and buffer types.

This application note presents an evaluation of the Flexsafe® Pro Mixer's performance in dissolving Tris, Dulbecco's Phosphate-Buffered Saline (DPBS), and sodium citrate buffers using 5, 10, and 20 L bags. By analyzing conductivity and visual mixing data, this study aims to demonstrate the mixer's capability to achieve rapid and consistent buffer dissolution, enhancing productivity and ensuring high-quality outcomes in biopharmaceutical manufacturing.

Materials

Reagents
Deionized (DI) water

Buffers
Buffers used in this study are shown in Table 1.

Table 1: *Type, Concentration, and Amount of Buffers Used*

Buffer	Material	Final Concentration [g/L]	Amount for 20 L [kg]	Amount for 10 L [kg]	Amount for 5 L [kg]
Tris	Sodium chloride	58.44	1.1688	0.5844	0.29220
	Tris base	6.06	0.1212	0.0606	0.03030
	Tris HCl	3.15	0.0630	0.0315	0.01575
DPBS	DPBS	9.55	0.1910	0.0955	0.04775
Sodium citrate	Citric acid	279.40	5.5880	2.7940	1.39700
	Sodium citrate	9.60	0.1920	0.0960	0.04800

Consumables
Flexsafe® Pro Mixer bags (5 , 10 L, and 20 L)

Equipment
Flexsafe® Pro Mixer 5 L | 10 L | 20 L (PRO-MIXER-SV), made of:
- Flexsafe® Pro Mixer Base Unit
- Flexsafe® Pro Mixer Bag Holder 5 L
- Flexsafe® Pro Mixer Bag Holder 10 L
- Flexsafe® Pro Mixer Bag Holder 20 L
- Conductometers
- Conductivity sensors

Methods

The hardware was installed on a weighing scale, and the bag was set up in the holder. The balance was tared, and the bag was filled with deionized (DI) water to 80% of its nominal volume. The impeller was run at specified speeds: 500 rpm for the 20 L bag and 400 rpm for the 5 and 10 L versions. The conductivity sensor was calibrated and placed in a corner of the bag, and time and conductivity were recorded. Solutes were added according to Table 1, with the addition time and method documented. "Mixing time 1" was defined as the time when 99% of the final conductivity value was reached, and all subsequent measurements remained within a 1% tolerance for at least 5 minutes.

Powder dissolution was visually checked, and the time when fully dissolved was noted, with mixing continued for at least two more minutes. The test was repeated twice more for a total of three repetitions. Data and videos were exported for analysis.

Results

The conductivity and visual inspection results are listed in Table 2.

Table 2: *Mixing Times for Different Flexsafe® Pro Mixer Bag Sizes and Buffers*

Bag Size	Solution	Speed [rpm]	Time to t99% Conductivity (All Trials) [mm:ss]	Time to t99% Conductivity (Average) [mm:ss]	Time to Complete Visual Mixing (Average) [mm:ss]
5 L	Tris-buffer	400	00:30 00:50 01:00	00:47	02:50
	DPBS	400	00:40 00:50 01:00	00:50	01:00
	Sodium citrate	400	01:19 02:40 01:39	01:53	03:40
10 L	Tris-buffer	400	00:50 01:30 01:30	01:17	02:50
	DPBS	400	00:30 01:10 00:50	00:50	02:20
	Sodium citrate	400	01:50 02:00 02:00	01:57	03:00
20 L	Tris-buffer	500	01:50 00:40 01:30	01:20	03:00
	DPBS	500	01:10 01:20 01:20	01:17	04:20
	Sodium citrate	500	02:49 01:50 02:10	02:16	05:00

Process data for mixing Tris buffers in 5 L Flexsafe® Pro Mixer bags shows conductivity reached the maximum value at the corner of the bag in around 30 seconds (Figure 1), which is the time needed for fully loading the powder. Mixing was visually confirmed to be complete (the solution was clear and there were no visible particles of powder) in under 3 minutes. An example is shown in Figure 2.

Figure 1: *Tris Buffer Preparation in a 5 L Flexsafe® Pro Mixer Bag*

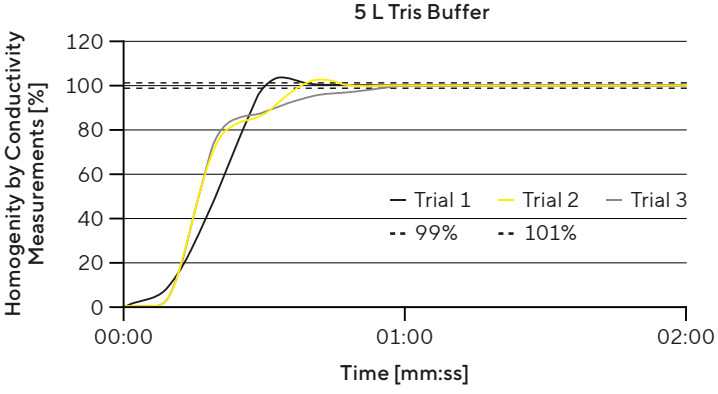
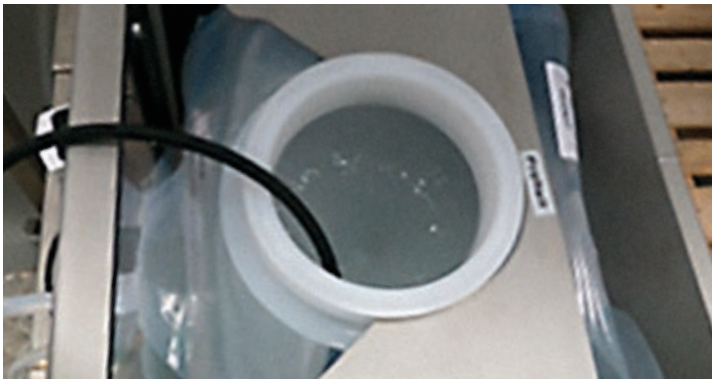
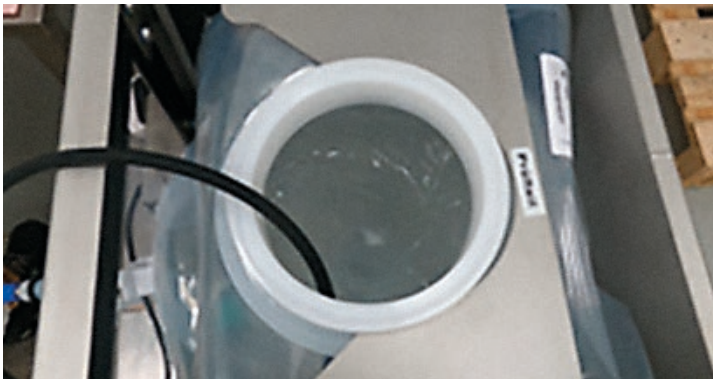


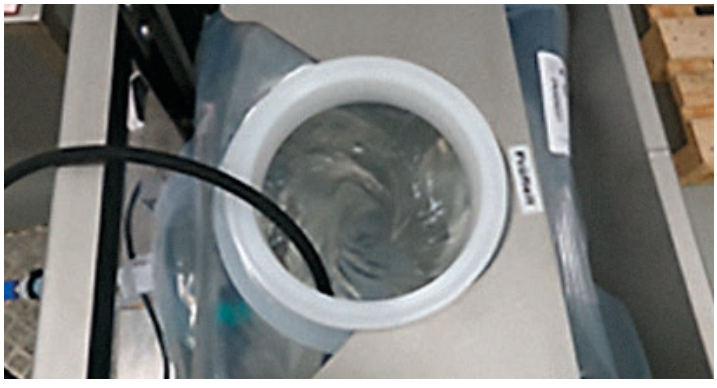
Figure 2: *Visual Inspection of Powder Dissolution After* ***(A)*** *30 Seconds,* **(B)** *1 Minutes, and* **(C)** *3 Minutes*



A Tris buffers fully loaded (30 s)



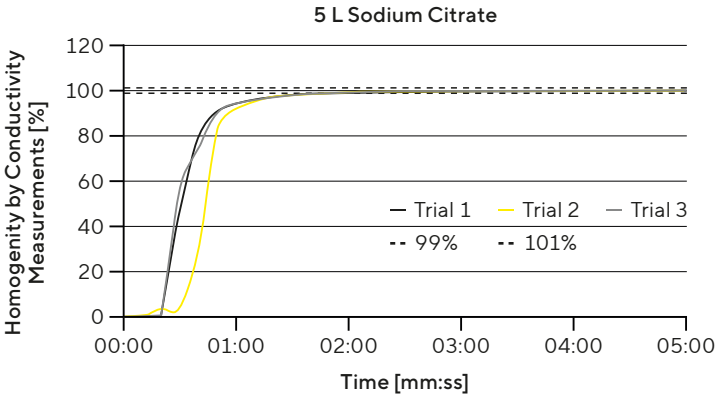
B Tris buffers during mixing (1 min)



C Tris buffers mixed by visual (3 min)

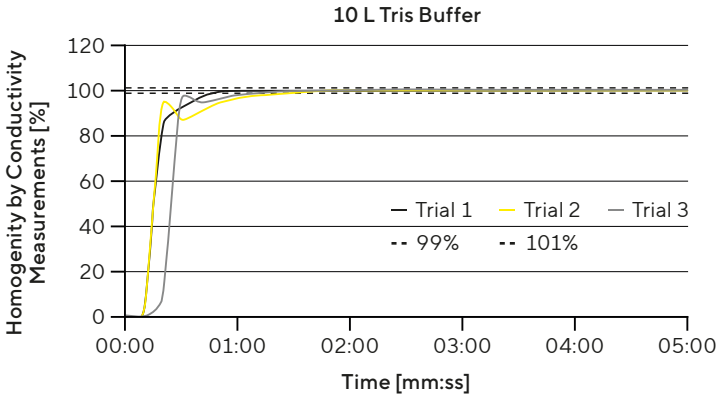
Process data for mixing sodium citrate in 5 L Flexsafe® Pro Mixer bags shows conductivity reached the maximum value at the corner of the bag after 1 minute 19 seconds to 2 minutes 40 seconds (Figure 3). Mixing was visually confirmed to be complete in under 3–4 minutes.

Figure 3: *Sodium Citrate Preparation in 5 L Flexsafe® Pro Mixer Bags*



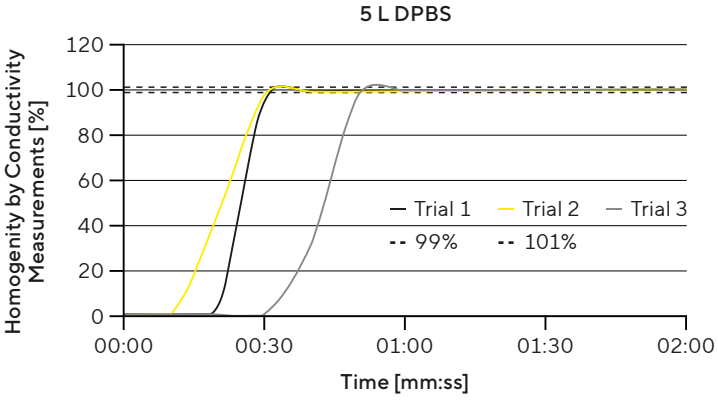
Process data for mixing Tris buffers in 10 L Flexsafe® bags shows conductivity reached a maximum value at the corner of the bag in around 1 minute 30 seconds (Figure 5). Mixing was visually confirmed to be complete in under 3 minutes.

Figure 5: *Tris Buffer Preparation in a 10 L Flexsafe® Pro Mixer Bag*



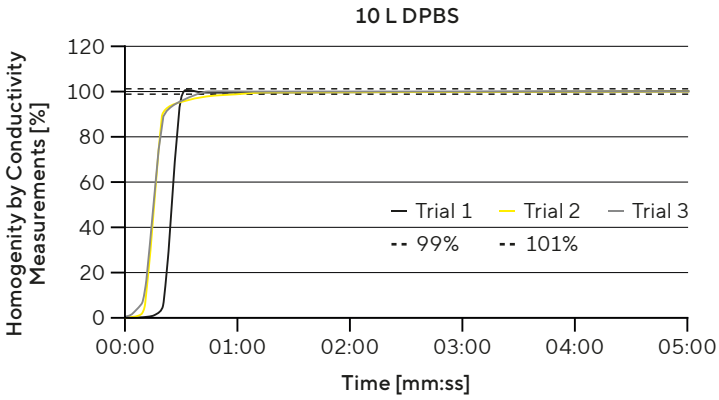
Process data for mixing DPBS in 5 L Flexsafe® Pro Mixer bags shows conductivity reaced its maximum value at the corner of the bag in around 30 to 45 seconds (Figure 4). Mixing was visually confirmed to be complete in under 1 minute.

Figure 4: *DPBS Preparation in a 5 L Flexsafe® Pro Mixer Bag*



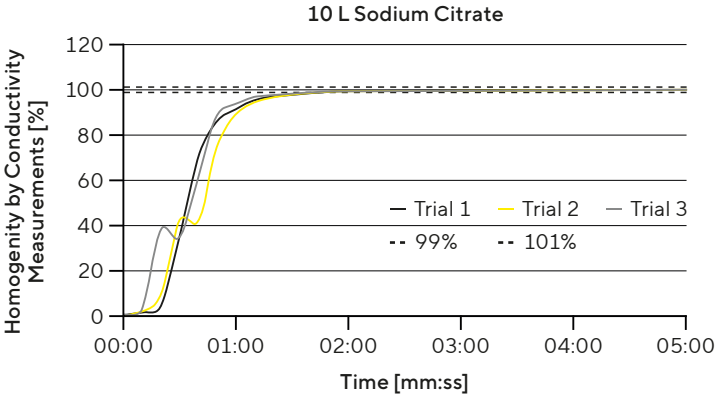
Process data for mixing DPBS in 10 L Flexsafe® Pro Mixer bags shows conductivity reached a maximum value at the corner of the bag in around 30 to 70 seconds (Figure 6). Mixing was visually confirmed to be complete in under 2 minutes 30 seconds.

Figure 6: *DPBS Preparation in a 10 L Flexsafe® Pro Mixer Bag*



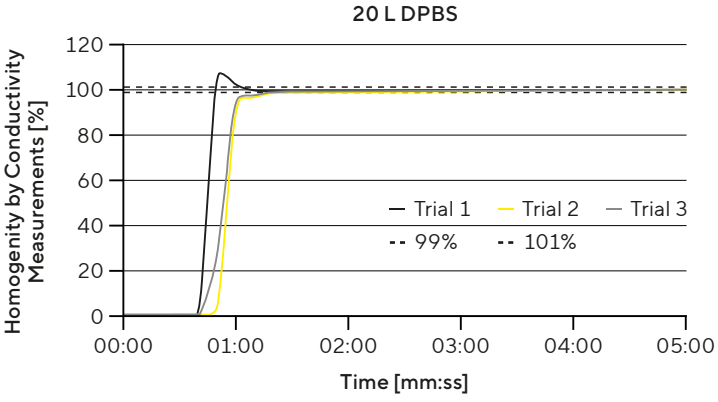
Process data for mixing sodium citrate for 10 L Flexsafe® Pro Mixer bags shows conductivity reached a maximum value at the corner of the bag in around 1 minute 50 seconds to 2 mins (Figure 7). Mixing was visually confirmed to be complete in under 3 minutes.

Figure 7: *Sodium Citrate Preparation in a 10 L Flexsafe® Pro Mixer Bag*



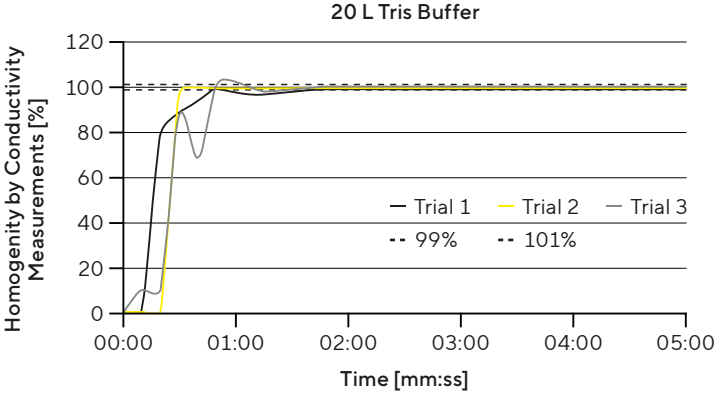
Process data for mixing DPBS in 20 L Flexsafe® Pro Mixer bags shows conductivity reached a maximum value at the corner of the bag in under 1 minute 20 seconds (Figure 9). Mixing was visually confirmed to be complete in under 5 minutes for the worst case.

Figure 9: *DPBS Preparation in a 20 L Flexsafe® Pro Mixer Bag*



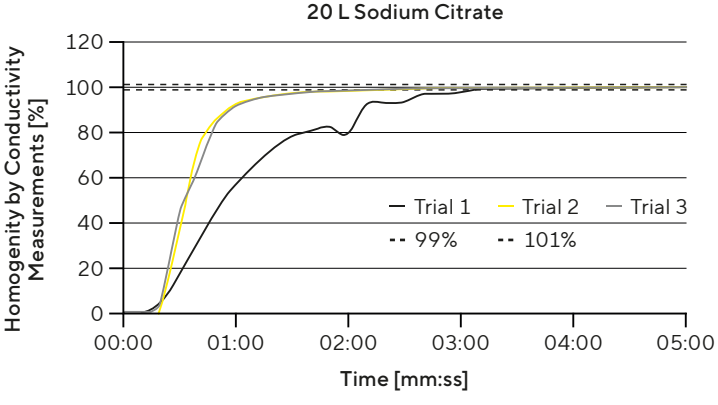
Process data for mixing Tris buffers for 20 L Flexsafe® Pro Mixer bags shows conductivity reached a maximum value at the corner of the bag in under 1 minute 50 seconds for the worst case (Figure 8). Mixing was visually confirmed to be complete in under 3 minutes.

Figure 8: *Tris Buffer Preparation in a 20 L Flexsafe® Pro Mixer Bag*



Process data for mixing sodium citrate in 20 L Flexsafe® Pro Mixer bags shows conductivity reached a maximum value at the corner of the bag in around 1 minute 50 seconds to 2 minutes 49 seconds (Figure 10). Mixing was visually confirmed to be complete in under 5 minutes.

Figure 10: *Sodium Citrate Preparation in a 20 L Flexsafe® Pro Mixer Bag*



Discussion

The Flexsafe® Pro Mixer demonstrates significant efficiency and reliability in buffer preparation across various volumes and buffer types. The results indicate that the mixer achieves rapid and consistent dissolution of powders, with conductivity reaching 99% of its final value swiftly across all tested bag sizes and buffer types. For instance, Tris buffer prepared in the 5 L bag reached t99% conductivity in approximately 30 seconds, while the sodium citrate buffer prepared in the 20 L bag took slightly longer, up to 2 minutes and 49 seconds. This variability highlights the mixer's adaptability to different chemical properties and volumes.

The visual inspection corroborates the conductivity data, confirming the complete dissolution of powders with no visible particles within a few minutes. No foaming or splashing effects were observed during all the trials.

In biopharmaceutical processes, these buffers play critical roles in various stages. For example, Tris buffer is often used in protein purification processes, where maintaining a stable pH is essential for protein stability and activity. DPBS is commonly used in cell culture applications to maintain osmotic balance and provide essential ions for cell metabolism. Sodium citrate is used in the formulation of certain pharmaceutical products, where it serves as a buffering agent to maintain the stability and solubility of active ingredients. It is also used in the preparation of vaccines, where it helps to stabilize the pH and enhance the shelf-life of the vaccine formulations.

These findings have substantial implications for biopharmaceutical applications where precise buffer preparation is critical. The Flexsafe® Pro Mixer can enhance productivity by reducing preparation times and ensuring consistent buffer quality, which is essential for downstream processes.

Conclusion

The Flexsafe® Pro Mixer is a versatile and efficient tool for buffer preparation, offering rapid dissolution and consistent mixing across different volumes and buffer types. The inclusion of buffers such as Tris, DPBS, and sodium citrate in critical process steps like protein purification, cell culture, and vaccine formulation, underscores the importance of reliable buffer preparation. The Flexsafe® Pro Mixer stands out as a promising solution for improving buffer preparation processes, potentially leading to enhanced productivity and quality in biopharmaceutical manufacturing.

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