



Application Note

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Flexsafe® Pro Mixer

The Fast, Flexible and Intelligent Solution for 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 50 L to 1,000 L using five buffer solutions: Glycine buffer, Tris buffer, 20x DPBS, Sodium Citrate and Sodium sulfate | MES. These buffers are commonly used in different steps of biomanufacturing such as tangential flow filtration, pH re-equilibration, and final formulation. The powders used to manufacture those buffers are mainly sinking powders and some of them are high concentrated buffers.

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 dissolve powders in less than 5 minutes in most tested cases and in less than 20 minutes for the worse case.

Introduction

The purpose of this application study is to assess the performance of the single-use Pro Mixer used in five different buffer preparations. The 20x DPBS is a buffer solution often used in downstream processes to maintain a physiological pH range during protein purification, filtration or final formulation. Tris buffer is commonly used in pH equilibration, Tangential Flow Filtration and final formulation. Glycine buffer is commonly used as an elution buffer for protein A columns and Sodium Citrate as an elution buffer. Sodium Sulfate is difficult to dissolve and constitutes a worst case in our study.

To this end, several powders are mixed and dissolved according to concentrations listed in table 1.

The procedure for buffer preparation includes the incorporation of the powders in the bag partially filled with WFI 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 1.

The mixing technology selected for this application is Flexsafe® for Pro Mixer with volumes of 50 L, 100 L, 200 L, 400 L, 650 L and 1,000 L.

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 buffer salts. In order to work under customer conditions, the speed has been adjusted depending on the final volume to avoid foaming effects or splashing (see table 2).

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 (50 L, 200 L, 400 L, 650 L and 1,000 L) including single-use pH and conductivity sensors
- Powder bags (15 L and 30 L)
- Powders (listed in table 1).
- Deionized water

Buffer	Material	Final Concentration (g/L)	Amount of powder added (kg) for a 200 L preparation
Glycine	Glycine	7.51	1.502
	Sodium Chloride	2.05	0.410
Tris	Sodium Chloride	58.44	11.69
	TRIS Base	6.06	1.212
20x DPBS	TRIS HCL	3.15	0.63
	DPBS without Ca and Mg	191	38.2
Sodium Citrate	Citric Acid	9.6	1.92
	Sodium Citrate	279.4	55.88
Sodium Sulfate MES	Sodium Sulfate	170.5	34.1
	MES, Free Acid	3.12	0.624
	MES Sodium Salt	1.95	0.390

Table 1: Buffer recipes given for 200 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 buffers are prepared in standard Flexsafe® Bags for Pro Mixer filled with deionized water to 80% of the final volume according to the experimental plan shown in table 2.

Buffer	Bag volume (impeller speed in rpm)					
	50 L (300)	100 L (400)	200 L (500)	400 L (725)	650 L (750)	1,000 L (750)
Glycine buffer			•			
Tris buffer	•	•	•	•	•	•
20x DPBS	•		•			•
Sodium Citrate	•		•			•
Sodium sulfate MES	•		•			

Table 2: Volumes tested per buffers and rotation speed used for each volume.

2. Impeller speed is set based on the nominal test volume to avoid foaming effects or splashing (see table 2).

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.

Results

1. 200 L Glycine Buffer Mixed in Less than 2 Minutes

The Glycine and sodium chloride added to make the Glycine buffer are dissolving readily leading to an almost instantaneous mixing of this buffer confirmed by visual inspection. In 200 L, this buffer is processed in less than 2 minutes including time for powder additions. The conductivity immediately changes at the addition of the Sodium Chloride.

Glycine 200 L

pH & conductivity	<2 min
Visual inspection	

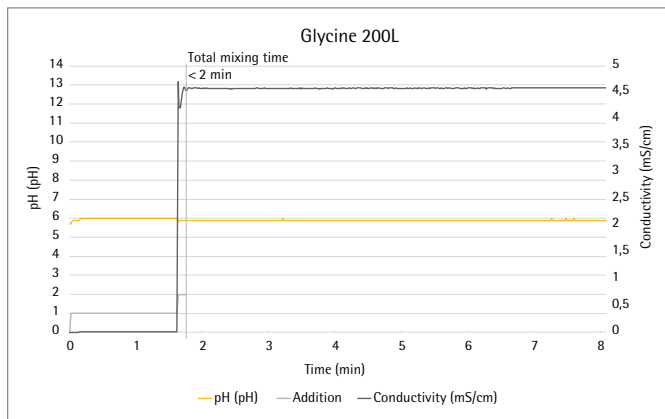


Fig. 1: Glycine buffer preparation in 200 L Flexsafe® Bag for Pro Mixer

2. Tris Buffer Mixed in Less than 5 Minutes throughout the Range from 50 L up to 1,000 L

Tris buffer is composed of Sodium Chloride, Tris base and Tris HCL which are mixing almost instantaneously. As with all sinking powders, a deep inspection is performed throughout the bag and especially in the four bag corners and this visual inspection confirms this fast dissolution performance. Mixing time is achieved in less than 5 minutes in the 50 L, 100 L, 200 L, 400 L, 650 L and 1,000 L.

Tris 50, 100, 200, 400, 650 & 1,000 L

pH & conductivity	<5 min
Visual inspection	

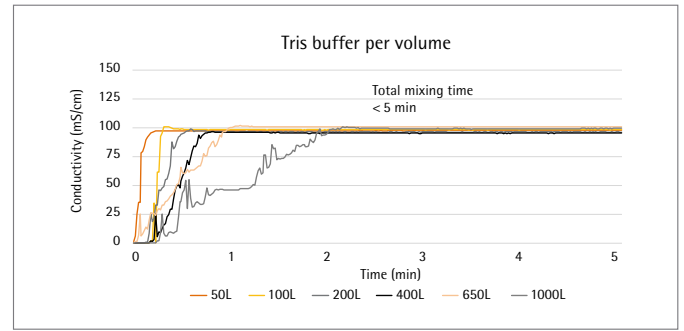


Fig. 2: Tris buffer preparation in 50 L, 100 L, 200 L, 400 L, 650 L and 1,000 L Flexsafe® Bag for Pro Mixer

3. High Concentration 20x DPBS Buffer Mixed in Less than 10 Minutes in 1,000 L

DPBS is prepared in high 20x concentration which represents a worse case in terms of final concentration and quantities of powder added. For example, 191 kg of DPBS were mixed with 800 L of water to prepare 1,000 L of 20x DPBS buffer. The addition of the 9 powder bags took around 9 minutes to deliver into the Flexsafe® Pro Mixer. Despite this, the Flexsafe® Pro Mixer was able to dissolve and mix this buffer in less than 5 minutes for the 50 L and the 200 L and in less than 10 minutes for the 1,000 L.

20x DPBS

Mixing Time Measurement	50 L	200 L	1,000 L
pH & conductivity	<5 min	<10 min	
Visual inspection			

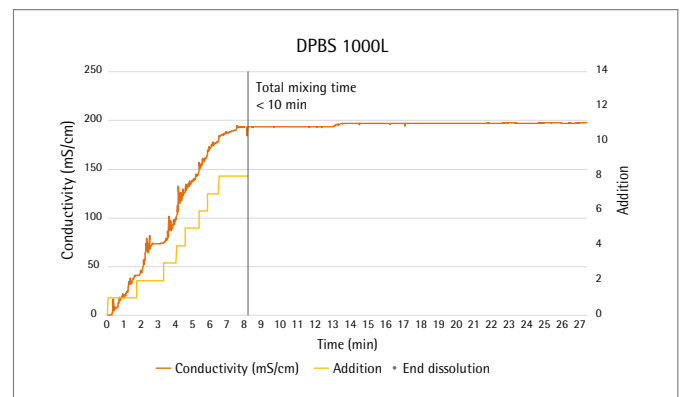


Fig. 3: 20x DPBS buffer preparation in 1,000 L Flexsafe® Bag for Pro Mixer

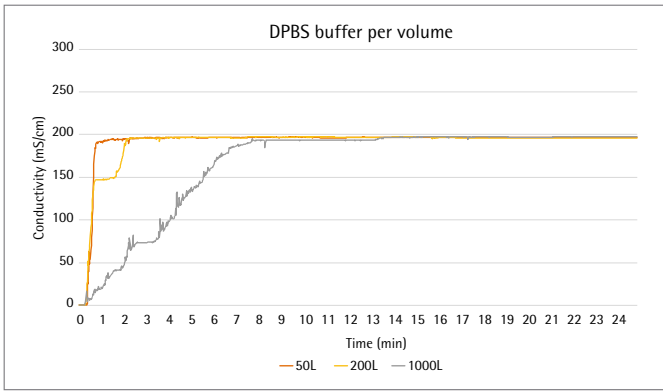


Fig. 4: 20x DPBS buffer preparation in 50 L, 200 L and 1,000 L Flexsafe® Bag for Pro Mixer

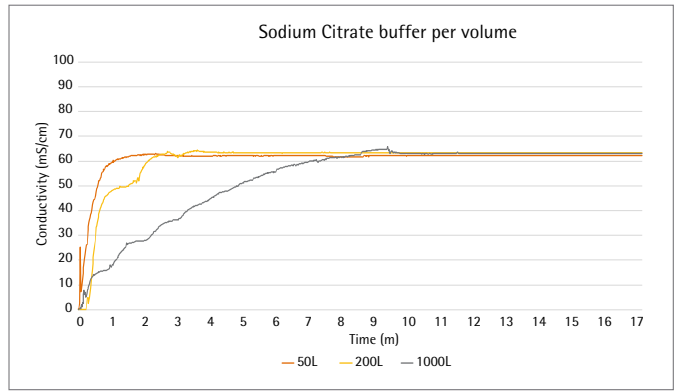


Fig. 6: Sodium Citrate buffer preparation in 50, 200 L and 1,000 L Flexsafe® Bag for Pro Mixer

4. 1,000 L Sodium Citrate Buffer Prepared with 290 kg of Powder in Less than 15 Minutes

Sodium Citrate buffer is also a difficult case as it consists in the addition of large amounts of powder. For the 1000 L, 290 kg of powder distributed in 14 bags (13 bags of Sodium Citrate and 1 bag of Citric acid) were mixed within 800 L of water. We can clearly observe on the graphs the drop of pH at the last addition of the Citric acid. Under this worst case conditions, the Flexsafe® Pro Mixer has demonstrated its ability to dissolve and mix this buffer in less than 5 minutes for the 50 L and the 200 L and less than 15 minutes for the 1000 L.

Sodium Citrate

Mixing Time Measurement	50 L	200 L	1,000 L
pH & conductivity	<5 min		<10 min
Visual inspection			<15 min

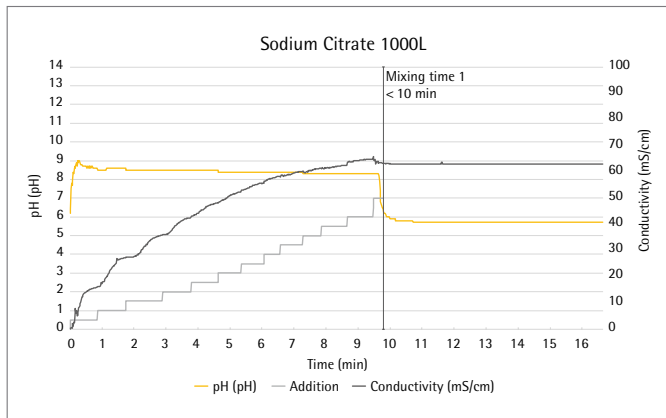


Fig. 5: Sodium Citrate buffer preparation in 1,000 L Flexsafe® Bag for Pro Mixer

5. Results for the Sodium Sulfate | MES Buffer

Sodium sulfate | MES buffer represents another worst case in terms of powder dissolution as it is known to form large and high-density clumps which are difficult to dissolve. We can clearly observe on the graph the pH variations at the additions of the MES free acid and MES Sodium. Despite the high concentration, quantities of powder added and clumps forming, the system was able to achieve a full mixing in less than 10 minutes for the 50 L and less than 20 minutes for the 200 L.

Sodium Sulfate | MES

Mixing Time Measurement	50 L	200 L
pH & conductivity	<10 min	<10 min
Visual inspection		<20 min

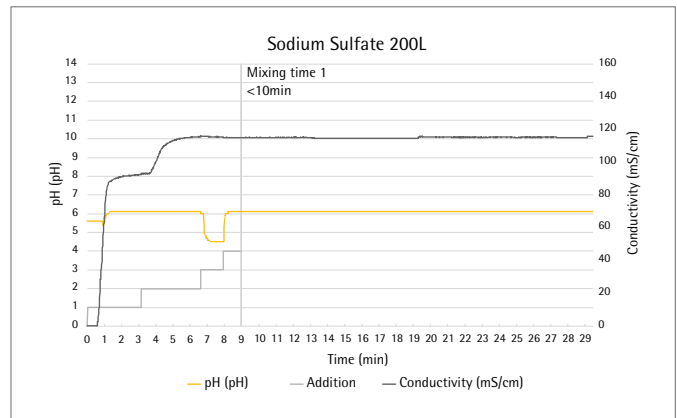


Fig. 7: Sodium sulfate | MES buffer preparation in 200 L Flexsafe® Bag for Pro Mixer

6. System Performance per Volume

6.1 50 L

The Flexsafe® Pro Mixer system was able to achieve a complete dissolution of all the different buffers in less than 5 minutes, except for the worse case, Sodium Sulfate | MES for which it took less than 10 minutes to obtain full dissolution checked by conductivity and visual inspection.

Buffer 50 L	Total Mixing Time
Tris buffer	<5 min
20x DPBS	
Sodium Citrate	
Sodium sulfate MES	<10 min

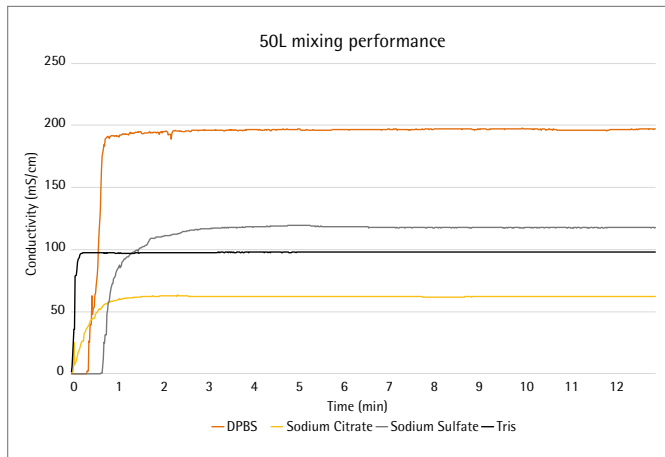


Fig. 8: 50 L Flexsafe® Pro Mixer performances

6.2 200 L

Again, the Flexsafe® Pro Mixer system was able to achieve a complete dissolution of all the different buffers in less than 5 minutes, except for the worse case, Sodium Sulfate | MES for which it took less than 10 minutes to obtain conductivity stabilization and less than 20 minutes to obtain full dissolution confirmed by visual inspection.

Buffer 200 L	Total Mixing Time
Glycine buffer	<5 min
Tris buffer	
20x DPBS	
Sodium Citrate	<20 min
Sodium sulfate MES	

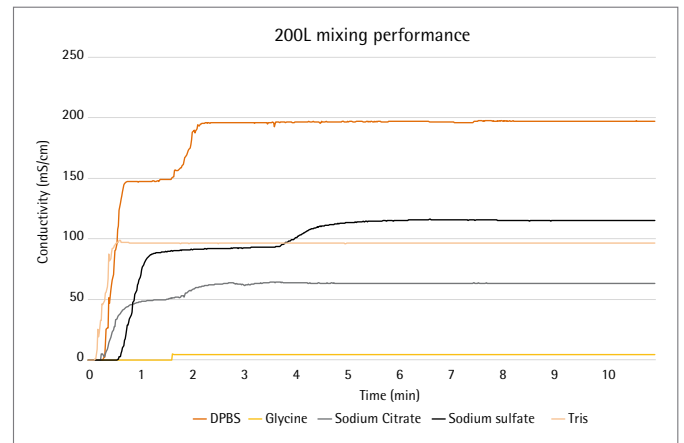


Fig. 9: 200 L Flexsafe® Pro Mixer performances

6.3 1,000 L

Even at 1000 L scale, the Flexsafe® Pro Mixer system was able to achieve a complete dissolution of the Tris buffer in less than 5 minutes. It took less than 10 minutes to fully dissolve the high concentration 20x DPBS. For the worse case, Sodium citrate, it took less than 10 minutes to obtain conductivity stabilization and less than 15 minutes to obtain full dissolution confirmed by visual inspection.

Buffer 1,000 L	Total Mixing Time
Tris buffer	<5 min
20x DPBS	<10 min
Sodium Citrate	<15 min

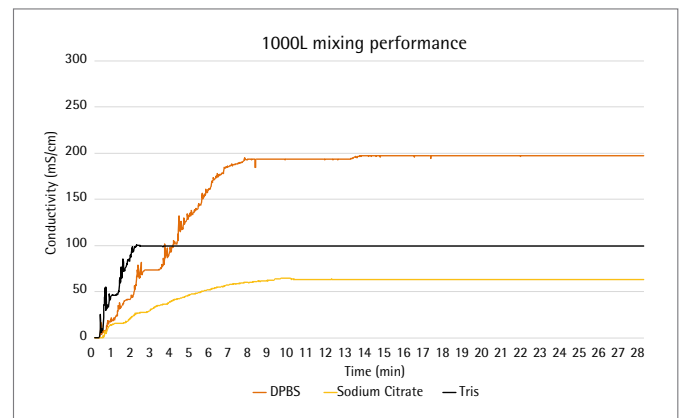


Fig. 10: 1,000 L Flexsafe® Pro Mixer performances

7. Summary of the Flexsafe® Pro Mixer performances

Buffer	Typical Application	Dissolution Characteristic	Mixing Time Measurement	Mixing Time per Volume		
				50 L	200 L	1,000 L
Glycine buffer	Elution buffer for protein A columns	Easy to dissolve	Conductivity & pH	-	<2 min	-
			Visual inspection			
Tris buffer	pH equilibration, Tangential Flow Filtration and final formulation	Common buffer used to test the 6 different volumes available between 50 L and 1000 L	Conductivity & pH	<5 min	<5 min	<5 min
			Visual inspection			
20x DPBS	Used in downstream processes to maintain a physiological pH range	High concentration solution	Conductivity & pH	<5 min	<5 min	<10 min
			Visual inspection			
Sodium Citrate	Elution buffer	High concentration and larger powder quantities inserted (290 kg in 1000 L)	Conductivity & pH	<5 min	<5 min	<10 min
			Visual inspection			<15 min
Sodium sulfate MES	Used in downstream processes when slightly acidic pH is required	Worse case: high concentration and known as forming difficult to dissolve, large and high-density clumps	Conductivity & pH	<10 min	<10 min	-
			Visual inspection	<10 min	<20 min	

Table 3: Summary of mixing time obtained with Pro Mixer System and main buffer characteristics in terms of easiness of preparation

Discussion

A rapid dissolution of the powder salts was observed in each case tested. The mixing time was found to be below 5 minutes in the majority of the cases, (11 out of 15), and a maximum mixing time below 20 minutes for the worse case (200 L Sodium sulfate | MES buffer).

For the low concentrated buffers, the dissolution is achieved almost right after adding the powder into the water. In such a case, the mixing time is essentially the time it takes to add the powders. This is a reason why high concentrated buffers were used to demonstrate the performance of the Pro Mixer.

For high concentration buffers, the Pro Mixer also demonstrated very good efficiency with mixing time below 5 minutes for the 20x DPBS and the Sodium citrate in 200 L.

For the worse case, 200 L Sodium sulfate | MES buffer, the Pro Mixer was able to achieve a complete dissolution in less than 20 minutes.

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, 14 bags have been added to prepare the 1,000 L Sodium Citrate buffer.

In the cases of 1,000 L sodium citrate and 200 L Sodium sulfate | MES, mixing was maintained until the solution became totally clear, this explains the difference in value between the conductivity data and the total mixing time obtained.

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, 5 to 10 minutes for a 1,000 L.

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 dissolve buffer powders even in worse case conditions such as high volume and high concentration, making the buffer 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® 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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