SARTORIUS

Application Note

September, 2022

Keywords or phrases:
Liquid Buffer Concentrates, Flexsafe® Pro Mixer, Glycine, TRIS, PBS, Batch Dilution

Liquid Buffer Concentrates Batch Dilution Using Flexsafe® Pro Mixer

Bastian Quaas[1], Johannes Borns[1], Maverick Lau[2], Clarisse Chinal[3]

[1] Sartorius Stedim Biotech, August-Spindler-Strasse 11, 37079 Goettingen, Germany

[2] Sartorius Singapore Pte Ltd1, 0 Science Park Road, The Alpha #02-13/14, Singapore Science Park I

[3] Sartorius Stedim FMT SAS, ZI des Paluds, Avenue de Jouques – CS 91051, 13781 Aubagne Cedex

Correspondence
Email: CellCultureMedia.EU@sartorius.com

Abstract

The mixing performances of the 200 L Flexsafe® Pro Mixer for batch dilution of various liquid buffer concentrates were evaluated by tracking the course, trend of pH and conductivity during the dilution process. Glycine, PBS, and TRIS liquid buffer concentrates were diluted three times to evaluate the reproducibility of the process. All batch dilution experiments were highly reproducible and all specifications for the obtained 1X buffers were met. Liquid buffer concentrates in combination with the single-use (SU) Flexsafe® Pro Mixer equipment can be used to produce large volumes of ready-to-use liquid buffers in a convenient manner while lowering the footprint. Liquid buffer concentrates also offer higher flexibility of volume preparation as compared with powder form which only allows for buffer preparation of a pre-determined and often excessive quantity.

Introduction

PBS, Glycine and TRIS buffers are commonly used in the downstream stages in biomanufacturing processes. PBS buffer can be prepared at a very high concentration of up to 20X in liquid form. It is used in cell culturing and in downstream processes to maintain a physiological pH range during protein purification and filtration. Glycine is typically used as an elution buffer for protein A chromatography at a buffering pH range of 1.5 to 3 while TRIS buffer is commonly used in downstream steps like pH equilibration for chromatography or Tangential Flow Filtration (TFF). Buffer concentrates can be easily diluted in batch mode prior to use and are also space-saving, also they can be used for in-line conditioning (ILC) or in-line dilution (ILD) in intensified downstream process. While buffers in powder form are easier to ship, they require more labor and are more prone to error during preparation.

Sartorius buffer concentrates are packaged in ready-to-use Flexsafe® bags. These concentrates can easily be diluted to a specific concentration using the SU Flexsafe® Pro Mixer system. The Flexsafe® Pro Mixer uses an off-center magnetic impeller assembly engineered to provide a powerful mixing of both liquid buffer concentrates and buffers in powder form at a large scale. The magnetic coupling of this impeller with the Flexsafe® Pro Mixer Drive Unit enables a rotation speed up to 500 rpm at these volumes.

Flexsafe® Pro Mixer was used to prepare common buffer formulations. Working concentrations of 1X 4Cell® PBS, 25 mM TRIS, 25 mM NaCl pH 7.2 and 100 mM Glycine pH 2.9 were achieved from diluting buffer concentrates. At 200 L scale, pH and conductivity targets were obtained in less than 430 seconds without adjustment.

Materials

Buffer Concentrates

Three different liquid concentrates representing the most commonly used buffers from chromatography equilibration, elution, and cell buffer, were tested (See Table 1).

Table 1: *Overview of Evaluated Buffer Concentrates and Their Composition.*

Buffer Name	Category	Chemical
20X PBS	Cell/Equilibration	KH_2PO_4
		Na_2HPO_4
		NaCl
18X Glycine	Elution buffer	Glycine
		Glycine-HCl
70X TRIS	Equilibration buffer	TRIS
		TRIS-HCl
		NaCl

Consumables

Table 2: *List of Consumables Used for the Application Experiments.*

Consumable	Lot	Amount	Comment
Flexsafe® Pro Mixer Bag 200 L	A0051662	3	For batch dilution

Equipment

Table 3: *List of Hardware Used for the Application Experiments.*

Name	Manufacturer
200 L Pro Mixer Pallet Tank	Sartorius
ProMixer Drive Unit	Sartorius
WM 520U Pump	Watson Marlow
Flowmeter HygienicMaster 300	ABB
pH-Meter	Knick
pH Electrode EasyFerm Plus	Hamilton
Conductivity meter	Knick
Conductivity sensor	Knick
Floor scale Combics 2	Sartorius
Laboratory Scale BP 4100	Sartorius
Laboratory Scale EA60EDE-I	Sartorius
Seven Go Duo Pro SG78	Mettler Toledo

Methods

Analytical methods

pH Measurement

The pH-meter was calibrated before each buffer batch dilution using appropriate calibration solutions. The measurement interval was set to 10 seconds. The pH was allowed to equilibrate for 5 mins in deionized water before addition of the buffer concentrate for 18X Glycine and 70 X TRIS/NaCl. The equilibration time for the 20X PBS batch dilution differed from batch to batch.

Conductivity Measurement

The conductivity-meter was calibrated before each buffer batch dilution using appropriate calibration solutions.
The measurement interval was set to 10 seconds. Batch dilutions were performed with deionized water. The temperature of the deionized water ranged from 19.7 to 22.7 °C. The conductivity was normalized to 25 °C by assuming an increase of conductivity of 2% per °C, as described in USP 644.

The measurement of samples after preparation of buffer concentrates and subsequent dilutions were analyzed at 25 °C without temperature compensation.

Results

Buffer Concentrates Parameters

Buffer concentrates were subjected to conductivity and pH measurements to ensure they are within standard specifications.

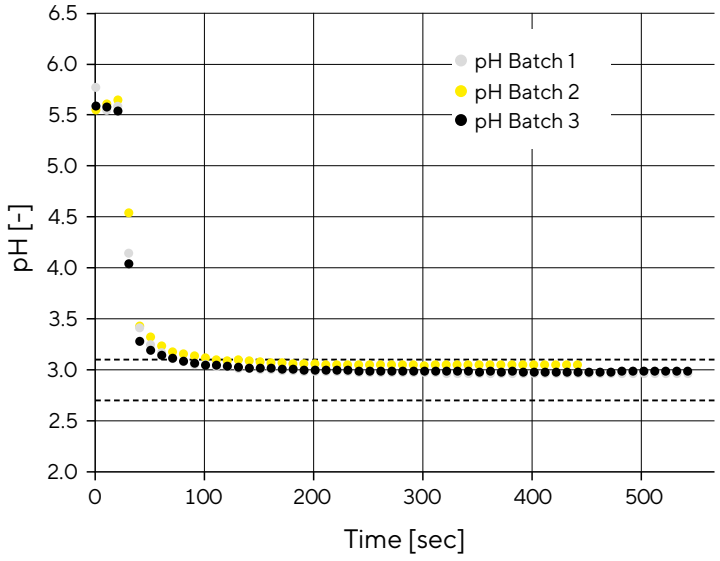
Table 4: *: Buffer Concentrates Conductivity and pH at 25 °C.*

Buffer Concentrate	pH	Conductivity [mS/cm]	T [°C]
18X Glycine buffer	2.83	33.0	25
20X Phosphate Buffered Saline	6.33	195.7	25
70X TRIS/NaCl buffer	7.07	119.2	25

Batch Dilution of 18X Glycine Concentrate

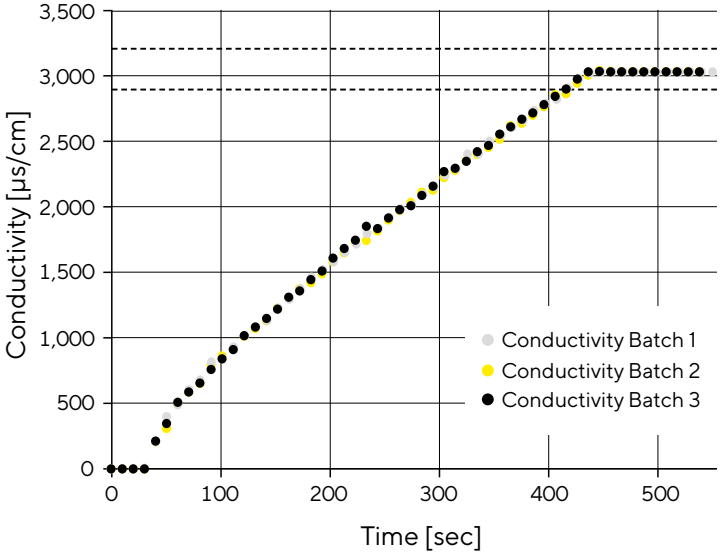
The pH trend has shown that the dilution process using the 200 L Pro Mixer equipment was highly reproducible (see Figure 1). The addition of the 18X Glycine concentrate started 20 seconds after initiating the pH/conductivity monitoring.
The pH-value dropped from pH 5.5 - 6 to below 3 within seconds after starting the addition of the 18X Glycine concentrate.
The pH-value slowly decreased further with progressing time and remained constant after the addition of buffer concentrate was finished after 430 seconds. All batches showed a similar trend and an end pH within the specification limits.

Figure 1: *pH Trend of 18X Glycine Dilution*



Note. The pH-trend of three batch dilutions of a 18X Glycine concentrate using a 200 L Pro Mixer equipment. Dotted lines represent the lower and upper specifications. The temperature of the diluted solutions ranged from 20 – 22 °C.

Figure 2: *Conductivity Trend of 18X Glycine Dilution*



Note. The conductivity-trend of three batch dilutions of a 18X Glycine concentrate using a 200 L Pro Mixer equipment. Dotted lines represent the lower and upper specifications. The temperature of the solutions ranged from 20 – 22 °C. The conductivity was normalized to 25 °C based on a 2% increase per °C.

The conductivity trend also showed that the batch dilution was highly reproducible within the three batches produced (see Figure 2). The addition of the buffer concentrate started about 20 seconds after the conductivity monitoring was initiated. After 30 seconds a linear increase of conductivity was observed until the concentrate addition was stopped after 430 seconds.

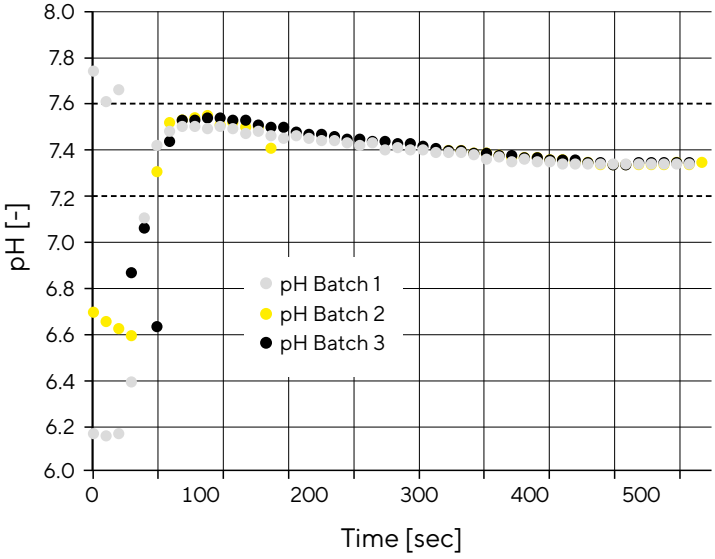
Afterwards the conductivity remained constant within the specification limits as expected. Overall, the batch dilution process took about 400 seconds to produce 200 L of 0.1 M Glycine buffer.

Batch Dilution of 20X Phosphate Buffered Saline Concentrate

The addition of concentrated buffer was started about 20 seconds after initiating the monitoring of pH. After about 50 seconds all batches showed a similar pH trend and the pH increased to about 7.5. Afterwards the pH decreased slowly and remained constant within the specification after the addition of 20X PBS was stopped at about 400 seconds. All three batches showed nearly no deviations in regard to the end pH value achieved.

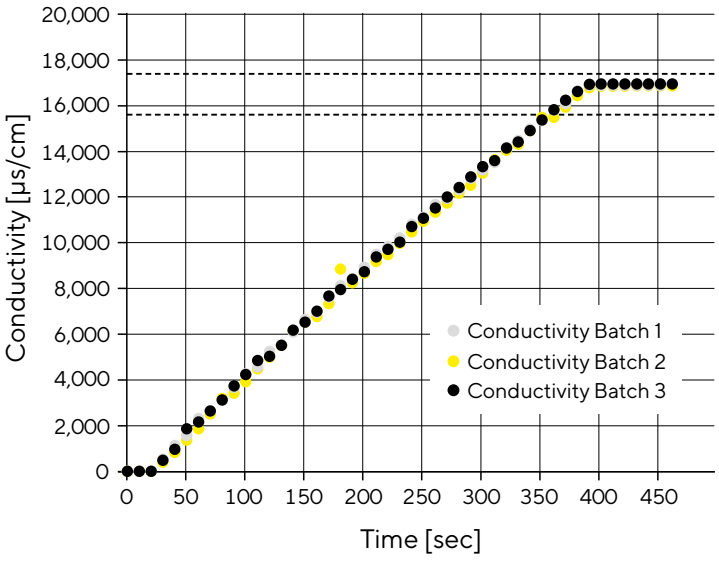
The pH before the addition of the 20X PBS differed from batch to batch. This was presumably caused by the different equilibration times of the pH sensor in deionized water before starting the addition of the buffer concentrate. The equilibration time of the pH sensor in deionized water is prolonged. This behavior was observed upon assessing the experimental data after performing the experiments. As a result, the equilibration time for the pH sensor was prolonged to 5 mins after inserting it to the deionized water for all remaining batch dilutions (18X Glycine, 70X TRIS/NaCl).

Figure 3: *pH Trend of 20X PBS Dilution*



Note. Shows the pH-trend of three batch dilutions of a 20X Phosphate Buffered Saline (PBS) concentrate using a 200 L Pro Mixer equipment. Dotted lines represent the lower and upper specifications. The temperature of the diluted solutions ranged from 19.7 - 22.5 °C.

Figure 4: *Conductivity Trend of 20X PBS Dilution*



Note. Shows the conductivity-trend of three batch dilutions of a 20X Phosphate Buffered Saline concentrate using a 200 L Pro Mixer equipment. Dotted lines represent the lower and upper specifications. The temperature of the diluted solutions ranged from 19.7 – 22.5 °C. The conductivity was normalized to 25 °C based on a 2% increase per °C.

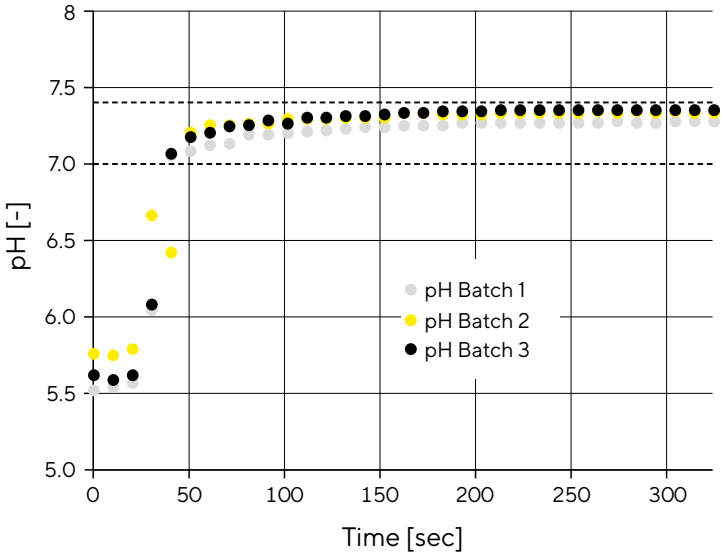
The conductivity trend showed that the batch dilution was highly reproducible within all three batches produced (see Figure 4). The addition of the buffer concentrate started about 20 seconds after the conductivity monitoring was initiated. After 30 seconds a linear increase of conductivity was observed until the addition was stopped at 400 seconds. Afterwards the conductivity remained constant within the specifications limits as expected. Overall, the dilution process for producing 200 L of 1X PBS buffer took about 370 seconds.

Batch Dilution of 70X TRIS/NaCl Concentrate

The pH trend shows that the dilution process using the 200 L Flexsafe® Pro Mixer equipment was highly reproducible (see Figure 5). The addition of the concentrate was started about 20 seconds after initating the pH monitoring. The pH value increased from pH 5.5 to 7.2 within 30 seconds after the addition of buffer concentrate. Afterwards, the pH increased slowly to about 7.4. The addition of the buffer concentrate was stopped at about 180 seconds and the pH remained stable as expected.

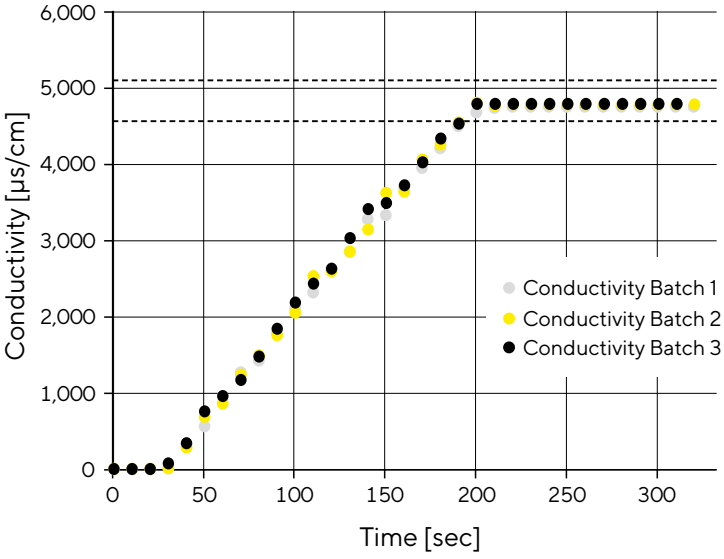
The pH remained within the specifications during the whole dilution process, although the pH was close to the upper specification at the end of the dilution process. This is because TRIS buffer is highly sensitive to temperature changes. Based on literature data, the pH decreases 0.03 units per 1 °C increase.

Figure 5: *pH Trend of 70X TRIS/NaCl Dilution*



Note. Shows the pH-trend of three batch dilutions of a 70X TRIS/NaCl concentrate using a 200 L Pro Mixer equipment. Dotted lines represent the lower and upper specifications. The temperature of the diluted solutions ranged from 20.2 - 21.9 °C.

Figure 6: *Conductivity Trend of 70X TRIS/NaCl Dilution*



Note. Shows the conductivity-trend of three batch dilutions of a 70X TRIS/NaCl concentrate using a 200 L Pro Mixer equipment. Dotted lines represent the lower and upper specifications. The temperature of the diluted solutions ranged from 20.2-21.9 °C.

During the pH monitoring, the temperature of the diluted buffer was between 20.2 and 21.9 °C, while the specification measurement was carried at 25 °C. Taking the temperature differences into account the pH of the batch dilutions would range from 7.21 to 7.22, which is well aligned with the results obtained from samples taken after the finished dilution process, which were measured at 25 °C (see Table 5).

The conductivity trend shows, that the batch dilution was highly reproducible within the three batches produced (see Figure 6). The addition of the buffer concentrate started about 20 sec after the conductivity monitoring has begun.

After 30 seconds a linear increase of conductivity was observed until the addition of concentrate was stopped at about 200 seconds. Afterwards the conductivity remained constant within the specifications limits as expected. Overall, only 2.86 L of the 70X concentrate was needed to produce 200 L of the 1X buffer in less than 3 minutes.

Offline Analysis of Diluted Buffers

After completing the batch dilution, 50 mL samples were taken and analyzed offline at 25 °C using a handheld pH/conductivity meter. Our study showed that the batch dilution approach using the single-use Pro Mixer equipment was highly reproducible and that all measured values fall within expected specifications.

Table 5: *: Results of Offline Analysis of Samples Derived From Batch Dilution at 25 °C*

Sample	Batch	pH Value	Mean pH	CV [%]	Conductivity	Conductivity Mean	CV [%]
1X Glycine	1	2.9	2.90	0.16	3.03	3.04	0.38
	2	2.89			3.05		
	3	2.9			3.03		
1X PBS	1	7.28	7.29	0.16	16.74	16.84	0.57
	2	7.3			16.85		
	3	7.28			16.93		
1X TRIS/NaCl	1	7.21	7.19	0.21	4.78	4.78	0.15
	2	7.18			4.79		
	3	7.19			4.78		

Discussion

Obtained results clearly show that high accuracy and reproducibility can be achieved utilizing liquid buffer concentrates in combination with the Pro Mixer equipment to prepare ready-to-use buffers. Buffers with extremely high concentration (70X) can also be diluted accurately to 1X using the Pro Mixer equipment. Thus, liquid buffer concentrates in combination with the Pro Mixer equipment can produce large volumes of ready-to-use buffers in a convenient and reliable manner. It also contributes greatly to space-saving as compared to storing 1X buffer long-term. While buffer in powder format is an excellent option for long term storage and ease of shipment, the additional handling required for preparation introduces additional risk of error and safety hazard. By using ready-to-use liquid buffer concentrates, manual handling is greatly reduced. Optionally, a sterile buffer preparation can be achieved by filling the needed Water for injection (WFI)-quality water through a sterile filter and by connecting the sterile buffer concentrate to the top lines of the pro mixer e.g. by using using the bio-welder equipment.

In hydration of buffer powder, many variables must be considered. Time for powder preparation, set-up, mixing, and powder bag change over is required. Ready-to-use liquid buffer concentrates eliminate several steps and are more efficient and flexible allowing for only the desired quantity of buffer to be manufactured.

In fact, the powder bag changeover may even take longer than the time necessary to reach conductivity stabilization at each additional step. Where else with ready-to-use liquid buffer concentrate bags, much less preparation time is needed, while also offering high flexibility of only diluting required volumes of 1X buffer.

Conclusion

This application note demonstrates the ability of the SU Flexsafe® Pro Mixer to prepare buffers from liquid concentrates with high accuracy and reproducibility. Using liquid buffer concentrates in ready-to-use bags has many advantages for consideration when selecting a buffer product type that fits your needs best.

Germany
Sartorius Stedim Biotech GmbH
August-Spindler-Strasse 11
37079 Goettingen
Phone +49 551 308 0

USA
Sartorius Stedim North America Inc.
565 Johnson Avenue
Bohemia, NY 11716
Toll-Free +1 800 368 7178

For more information, visit
www.sartorius.com

Status: 11 | 2022