Integrated Buffer Delivery and Management Utilizing Buffer Concentrates and an Automated Dilution System

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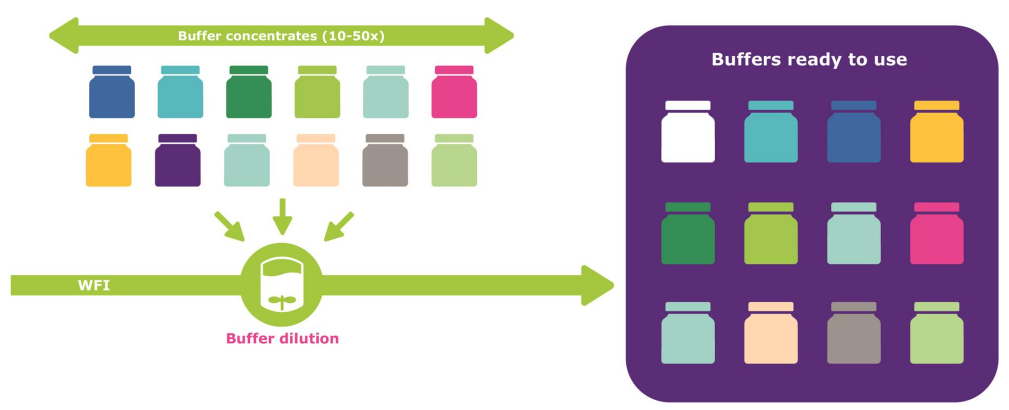
Introduction

Biopharmaceutical manufacturing is evolving in response to market pressures including rapid market expansion, continued uncertainty, new therapeutic modalities and cost pressure. In response, there has been significant emphasis on process intensification and productivity across unit operations, with more recent focus on increasing facility productivity. One such opportunity for facility productivity is centered around buffer preparation.

Traditional buffer preparation from powder requires multiple steps and typical pain points include: large amounts of classified floor space, significant labor requirements, intensive quality control testing, and excess capacity to account for preparation error.



Alternatives to traditional buffer preparation include ready to use buffers, buffer stock blending, and buffer dilution. Buffer dilution can answer these pain points through the combination of buffer concentrates (up to 50X), a buffer dilution skid that prepares ready to use solutions with accuracy and precision, and single use components for distribution throughout the plant. This combination can increase plant flexibility, while reducing classified floor space, labor and CAPEX requirements.

This work evaluates a buffer dilution skid that utilizes volumetric flow to accurately and precisely prepare ready to use buffers from concentrates.

Experimental

Buffer Dilution 17 System was utilized for this analysis. For accuracy and precision, recommended flow rates are: 1. minimum system flow rate of 5X the minimum flow rate of the largest pump, 2. Minimum pump flow rate of 10X the minimum flow rate, with an exception for a 50X dilution, where this minimum is not required.

For all experiments presented, the flow rates and totals were measured by tracking the mass of all solution source and collection vessels. All pH and conductivity measurements were taken using offline instruments on solution pools greater than 10 liters. All flow accuracy tests were run for at least 5 minutes after an initial flush of 5 liters was sent to waste and not collected. For concentrate evaluation the first 5 liters were sent to waste an then 10 liters were collected for offline evaluation of pH and conductivity. All tests were performed utilizing the system's programmed dilution sequences and thus the system was not manually operated.

System Evaluation

To assess system performance, flow accuracy was measured at the minimum and maximum flow rates across a variety of dilution factors and one intermediate flow rate. Flow rates evaluated are shown in Table 1.

System Flow (L/min)	Dilution Ratio			
	5X	10X	20X	50X
3.8	✓	✕	✕	✕
6.0	✱	✓	✕	✕
12.6	✱	✱	✓	✕
14.8	✱	✱	✓	✓
17.0	✓	✓	✓	✓

Table 1. Dilution ratios evaluated for different flow rates (✓), Dilution ratios and flow rates accessible, but not evaluated(✱) and Flow rates outside recommended flow rates(✕).

Results for flow accuracy are shown in Table 2. These results indicate less than one percent error for all conditions tested with the exception of the 50X dilution with a 14.8 L/min flow rate. This flow rate is at the edge of the minimum flow rate for a 50X dilution and additional testing or a longer evaluation time may have lowered the observed error.

System Flow (L/min)	5X Dilution		10X Dilution		20X Dilution		50X Dilution	
	Diluent Pump (% error)	Conc. Pump (% error)	Diluent Pump (% error)	Conc. Pump (% error)	Diluent Pump (% error)	Conc. Pump (% error)	Diluent Pump (% error)	Conc. Pump (% error)
3.8	0.55	0.30						
6.0			0.00	0.50				
12.6					0.08	0.16		
14.8					0.14	0.14	2.90	2.83
17.0	0.00	0.38	0.13	0.18	0.11	0.19	0.84	1.18

Table 2. Observed percent error for different dilution ratios and flow rates shown for both the diluent and concentrate pumps.

Concentrate Evaluation

Understanding the accuracy of the flow rate delivery, additional evaluation was undertaken with a 1 M Sodium Phosphate solution (Sigma-Aldrich, P3619) at various dilution ratios to evaluate performance. Similarly to the system evaluation, this testing evaluated the high and low flow rates for each dilution ratio, with the exception of 50X where only the maximum flow rate was evaluated (Table 3).

System Flow (L/min)	Dilution Ratio			
	5X	10X	20X	50X
3.8	✓			
6.0		✓		
12.6			✓	
17.0	✓	✓	✓	✓

Table 3. Dilution ratios and system flow rates evaluated for 1M Sodium Phosphate (✓)

Dilution of a 1M sodium phosphate solution was used to demonstrate the accuracy of the system as compared to manually prepared solutions under the conditions outlined in Table 4. When comparing the results from low and high flow rates within a given dilution ratio, pH and conductivity are consistent and less than 0.1 pH unit or 0.5 mS.

System Flow (L/min)	5X Dilution		10X Dilution		20X Dilution		50X Dilution	
	pH	Cond (mS)	pH	Cond (mS)	pH	Cond (mS)	pH	Cond (mS)
3.8	7.37	25.9						
6.0			7.38	14.75				
12.6					7.40	8.40		
17.0	7.36	25.9	7.37	14.73	7.36	8.39	7.35	4.03

Table 4. Dilution of a 1M sodium phosphate solution at the identified flow rates and dilution factors.

Buffer Production

To assess system performance to deliver ready to use solutions, six process relevant buffers were prepared from concentrate. These concentrate buffers were specifically designed to provide process pH and conductivity values at the desired dilution ratios and can be customized to an end user's specification. The evaluated buffers are often found in wash, rinse, elution and equilibration steps and challenged the performance of the system with a wide range in conductivity (1M to 5M) and dilution (3X to 40X) and produced buffers of varying pH, conductivity, and molarity.

Target Buffer	Dilution Ratio (X)	System Flow (L/min)	System Dilution		Manual Dilution	
			pH	Cond. (mS)	pH	Cond. (mS)
1 M Tris Base, pH 8.4	3	8.2	8.32	19.6	8.22	19.5
		12.6	8.32	19.3	8.26	19.4
		17.0	8.30	19.2	8.27	19.6
0.1 M Sodium Acetate, 0.5 M Sodium Chloride, pH 5.5	8	8.2	5.51	50.9	5.46	51.1
		12.6	5.50	51.2	5.48	50.7
		17	5.52	51.5	5.49	51.1
0.1 M Sodium Citrate, pH 5.4	15	8.2	5.41	19.0	5.41	19.0
		12.6	5.43	19.2	5.42	18.8
		17.0	5.43	19.2	5.42	18.9

Table 5. Comparison of Buffer dilution preparation from concentrates to ready to use solutions using an automated buffer dilution system or traditional manual buffer prep

Table 5 outlines the results of system and manual 3X dilution of a 3M Tris base to prepare a 1M Tris Base, pH 8.4 solution, an 8X dilution of 0.8M Sodium Acetate, 4.0 M Sodium Chloride to prepare a 0.1 M Sodium Acetate, 0.5 M Sodium Chrloride pH 5.5 solution and a 15X dilution of 1.5 M Sodium Citrate to prepare a 0.1 M Sodium Citrate, pH 5.4 solution across a variety of flow rates. Comparison of pH and conductivity between methods of preparation show consistent dilution to reach the targeted end points with only small variation between runs and methods. Temperature was not controlled for and this may account for the subtle offset in the resulting Tris buffer pH values. In addition, variation within preparation methods is similar if not somewhat more consistent for the system dilution.

Three additional buffers at higher concentration were evaluated including a 30X dilution of 1.5M Tris Acetate, 1.5 M Sodium Chloride to provide 50 mM Tris acetate, 50 mM Sodium Chloride pH 8, a 40X dilution of 2M citric acid for a 50 mM citric acid, pH 3.2 and a 40X dilution of 2M Tris to provide a 50 mM Tris, pH 8. Comparison of the system and manual dilutions are outlined in Table 6. Again, the largest differential is observed with Tris containing solutions, which may result from the lack of temperature compensation. It is possible that increased flush volumes at run initiation and increased run time would improve the accuracy and precision of the system dilutions. In this evaluation, short flush volumes (5 L) were used along with a minimum run volume of 10 liters.

Target Buffer	Dilution Ratio (X)	System Flow (L/min)	System Dilution		Manual Dilution	
			pH	Cond. (mS)	pH	Cond. (mS)
50 mM Tris Acetate, 50 mM Sodium Chloride pH 8.0	30	17	7.93	7.9	8.00	7.8
50 mM Citric Acid, pH 3.2	40	17	3.28	2.3	3.20	2.3
50 mM Tris, pH 8.0	40	17	7.28	3.6	7.42	3.5

Table 6 Comparison of Buffer dilution preparation from concentrates to ready to use solutions using an automated buffer dilution system or traditional manual buffer prep

Summary

Evaluation of a Buffer Delivery System using volumetric flow control shows excellent accuracy in buffer dilution.

This study evaluated the performance of a Buffer Delivery system across variable flow rates and dilution ratios and exhibits accuracy when flow rate is assessed.

System testing with a 1M Sodium Phosphate concentrate across these similar flow rate ranges and dilution ratios exhibits consistent pH and conductivity within specified dilution ratios.

Pairing the performance of the Buffer Delivery System with customizable concentrates, six process specific buffers were prepared at a variety of flow rates and dilution ratios. These results indicate the performance and preparation of ready to use buffers from concentrate with volumetric flow control without the need for manipulation post dilution.

Buffer preparation can be simplified through the use of premade buffer concentrates and a Buffer Delivery System that utilizes volumetric flow control for consistent and reliable performance.

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