Hydration and scale-up of AGT medium in HyPerforma and imPULSE Single-Use Mixers

Introduction

Cell culture media can have a significant impact on bioproduction output. Generally, cell culture media are available in either a ready-to-use liquid format or a dry format that must be hydrated before use. According to a 2014 study of manufacturing and market trends, dry media hydration was the most common method of media preparation, with dry-format media accounting for 90% of the media purchased by large-scale bioprocessing manufacturers [1]. The demand for dry-format media relates to their lower cost and storage footprint. Preparing dry media quickly and consistently can be critical to ensuring repeatable and robust cell growth and productivity.

Gibco™ Advanced Granulation Technology™ (AGT™) dry-format media are produced using a fluidized bed process, resulting in an easily solubilized granulated product that generates low dust formation. Most AGT media products are chemically defined, serum-free, animal origin–free buffered formulations that provide preadjusted final pH and osmolality [2]. These unique product characteristics help customers overcome many of the common challenges associated with dry media preparation such as multiple hydration steps, pH adjustments, long hydration times, inconsistent batches, and clumping and floating of medium.

With its distinct benefits, it is beneficial to understand AGT media hydration across different commonly used single-use mixers (S.U.M.s) and specific best-practice recommendations. To address this, testing was conducted to evaluate the hydration of an AGT medium in two mixing studies with a variety of Thermo Scientific™ HyPerforma™ and imPULSE™ S.U.M.s. The first study evaluated how rapidly the AGT medium could be hydrated at various working volumes under predetermined maximum agitation conditions. The second study evaluated the best hydration practices for maintaining the final target pH of the AGT medium. The results of these studies help demonstrate the scalability of hydrating AGT media, outline mixer efficiencies, compare differences in mixing attributes between mixer types, and provide recommendations for best practices for AGT media hydration. This should facilitate optimal planning for media handling operations in the cell culture production process.

Materials and methods

Standard 50 L, 200 L, and 2,000 L HyPerforma S.U.M.s were used along with a standard 2,000 L imPULSE S.U.M. Standard Thermo Scientific™ BioProcess Containers (BPCs) for each vessel type were modified with sampling and probe ports at the top, middle, and bottom of the BPC. The Thermo Scientific™ imPULSE™ BPC was also equipped with a spray nozzle on the top of the BPC, to which a peristaltic pump was connected, creating a liquid recirculation loop. This pump was set to circulate water from the bottom of the BPC through the spray nozzle at a rate of ~10 L/min during all mixing studies performed with the 2,000 L imPULSE S.U.M.

Previously, agitation was tested in each mixer at various working volumes with deionized (DI) water to determine the manufacturer-recommended maximum stirring speed for each volume, with results shown in Table 1. The manufacturer-recommended maximum stirring speed was the point at which the highest possible agitation occurs without shaft wobbling in the HyPerforma S.U.M. and without excessive splashing in the imPULSE S.U.M. The results of the testing were utilized as the basis for determining the agitation rates used in both the rapid hydration and pH shift mitigation studies.

The conversion from rpm to power input was calculated using Equation 1 [3] and a power number of 2.1 for the stirred-tank vessels (HyPerforma S.U.M.s).

$$P = N_p \rho N_i^3 D_i^5 \qquad \text{(Equation 1)}$$

N_p: power number, ρ: density of mixture, N_i: stirring speed, D_i: impeller diameter.

Rapid hydration study

The purpose of the rapid hydration study was to identify how quickly AGT media could be hydrated at variable working volumes in standard S.U.M.s. Gibco™ Dynamis™ AGT™ Medium was used for testing in the 200 L and 2,000 L HyPerforma S.U.M.s, and a Gibco™ prototype AGT medium was used for testing in the 50 L HyPerforma and 2,000 L imPULSE S.U.M.s. Prior to testing, a small-scale mixing study was performed to confirm that both media hydrate in similar time frames.

In the rapid hydration study, three working volumes—full, 5:1 and 10:1—were evaluated in each of the vessels tested. The BPC in each condition was filled with deionized (DI) water at ambient temperature (19–25°C) to 90% of the working volume tested. For full-volume testing, probes for pH and conductivity were inserted into the top and bottom of the BPC, and online data were logged with the Touchscreen Console. For the 5:1 and 10:1 working volumes, probes were inserted only into the bottom probe belt of the BPC.

The mixer was set to the manufacturer-recommended maximum stirring speed (Table 1), with the manufacturer-recommended amount of AGT medium being added as quickly as possible to the top of the mixer (over a period of 1–2 minutes in the smaller S.U.M.s, and 3–4 minutes in the 2,000 L S.U.M.s). A timer was started as soon as all of the medium was added to the mixer. The rapid addition method was chosen to assess the capability of the S.U.M.s to hydrate AGT media in a worst-case scenario and to create a procedure that is repeatable. This method was used for all mixers at all volumes tested. The medium addition method was changed for the 2,000 L HyPerforma S.U.M. testing at 5:1 volume, after nonideal initial results were observed. The new medium addition method is explained in the results section describing rapid hydration at 5:1 volume.

Table 1. Manufacturer-recommended maximum stirring speeds determined for mixers tested at each working volume.

	Working volume		Manufacturer-recommended maximum stirring speed	
Mixer	**Ratio**	**Volume (L)**	**Power input (W/m³)**	**Rate (rpm or Hz)**
50 L HyPerforma S.U.M.	Full	50	582	356 rpm
	5:1	10	193	144 rpm
	10:1	5	283	130 rpm
200 L HyPerforma S.U.M.	Full	200	702	356 rpm
	5:1	40	382	170 rpm
	10:1	20	427	140 rpm
2,000 L HyPerforma S.U.M.	Full	2,000	204	350 rpm
	5:1	400	117	170 rpm
	10:1	200	100	128 rpm
2,000 L imPULSE S.U.M.	Full	2,000	–	2 Hz
	5:1	400	–	0.9 Hz
	10:1	200	–	0.66 Hz

During mixing, samples were taken as outlined in Table 2. At each time point, a total of three 10 mL samples were taken, one each from the top, middle, and bottom sampling ports for full-volume testing. A single 10 mL sample was taken from the bottom sampling port for 5:1 and 10:1 working volumes. Osmolality and glucose levels of the samples were tested offline with an osmometer for osmolality and a BioProfile™ FLEX2 Automated Cell Culture Analyzer for glucose levels. Due to the design of the experiment, there was a delay in taking samples to running samples on the analytical instruments. A T95 mixing time—when the measured value reaches 95% of the final stable value—was calculated for conductivity, glucose, and osmolality. The T95 mixing times were calculated based on the analytic reaching 95% of the final recorded stable value determined from the data collected during the last 10 minutes of mixing. The conductivity data were collected from the online probe, with glucose and osmolality data collected from offline test results obtained from samples.

Table 2. Sampling plan for rapid hydration and pH shift mitigation studies.

Total mixing time (min)	Sampling frequency
0 to 20	Every 1 min
20 to 40	Every 5 min
40 to 60	Every 10 min
60 to 70	Every 1 min

pH shift mitigation study

The overall goal of the pH shift mitigation study was to determine how pH drift could be minimized when mixing AGT medium at lower working volumes. In this study, the 200 L HyPerforma S.U.M. was evaluated at 5:1 working volume (40 L) with the methods mimicking those of the rapid hydration study, except using lower agitation rates. This allowed direct comparison of this study's results to the same mixer and volume conditions in the rapid hydration study where agitation rates were set to the manufacturer-recommended maximum stirring speed (Table 1). pH and conductivity probes were inserted into the lower probe belt of the BPC, and data were logged with the Touchscreen Console. The mixer was filled with DI water to 90% of the final working volume, and agitation of the solution was set at 100 W/m^3 (108 rpm), or 26% of the 382 W/m^3 (170 rpm) recommended maximum setting at the 5:1 volume (Table 1). This study was repeated in the same manner with an agitation rate of 200 W/m^3 (137 rpm), or 52% of the manufacturer-recommended maximum stirring speed. The AGT medium was added as quickly as possible to the top of the vessel, and a timer was started after all the medium was completely added. Samples were collected using the same methods described in Table 2. The osmolality and glucose levels of the samples were tested offline using the same instruments described in the previous rapid hydration section, and the results were recorded. The results from the two agitation rates were compared to the results obtained for the 200 L HyPerforma S.U.M. at 5:1 volume in the rapid hydration study.

Results

Rapid hydration study: full volume

Full-volume rapid hydration mixing at the manufacturer-recommended stirring speed (Table 1) for each of the four mixers demonstrated that the AGT medium was fully entrained in solution and reached T95 mixing times with minimal foaming in under 45 minutes with the worst-case practice of medium addition. The calculated T95 mixing times and pH levels after 70 minutes of mixing are listed in Table 3, with trends depicted in Figures 1–4. For all mixers tested, the pH at the end of 70 minutes of mixing fell within the range of 6.9 to 7.1. This is within the range commonly expected for cell culture media. Excessive upward pH drift was not observed when mixing the AGT medium at full volume with maximum agitation (Figure 4).

With rapid hydration and medium addition at full volume, the smaller 50 L and 200 L HyPerforma S.U.M.s followed similar trends reaching homogeneity, with both having T95 mixing times from 1 to 3 minutes for all analytics (Figures 1–3). The larger 2,000 L HyPerforma and imPULSE

Table 3. T95 mixing times and end-of-mixing pH data for all mixers tested using rapid hydration at full volume.

	T95 mixing time (min)			End mix
Mixer	Glucose	Osmolality	Conductivity	pH
50 L HyPerforma S.U.M.	3	3	1	7.06
200 L HyPerforma S.U.M.	2	2	2	6.94
2,000 L HyPerforma S.U.M.	45	35	44.5	6.95
2,000 L imPULSE S.U.M.	30	30	28	6.85

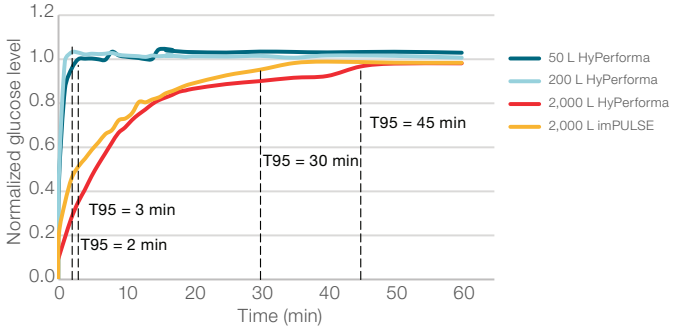

Figure 1. Normalized glucose levels from the full-volume rapid hydration study.

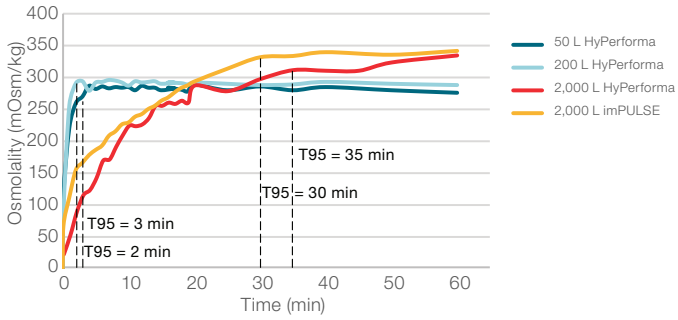

Figure 2. Osmolality from the full-volume rapid hydration study.

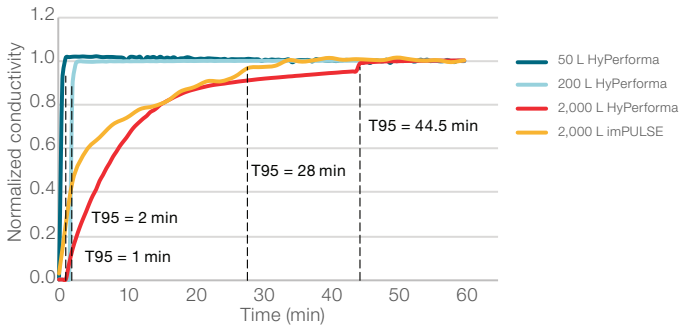

Figure 3. Normalized conductivity from the full-volume rapid hydration study.

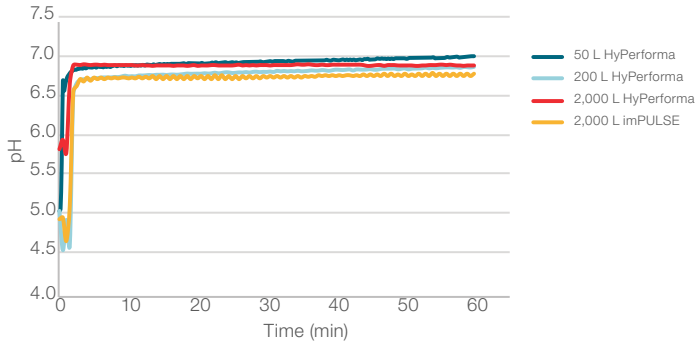

Figure 4. pH results from the full-volume rapid hydration study.

S.U.M.s showed similar hydration patterns as well, with T95 mixing times of 35–45 minutes in the HyPerforma S.U.M. and 28–30 minutes in the imPULSE S.U.M. (Figures 1–3). As was somewhat expected, hydration in the larger 2,000 L mixers occurred significantly more slowly compared to in the smaller 50 L and 200 L S.U.M.s. However, a factor contributing to the slower hydration time was the medium addition method. To replicate a worst-case scenario, the medium was added as rapidly as possible to the mixer, which allowed a large island and clumps of medium to form on the surface of the fluid. As anticipated, the rapid medium addition method was shown to be nonideal for hydrating large volumes of medium.

Rapid hydration study: 5:1 working volume

The rapid hydration of AGT medium was evaluated at the lower 5:1 working volume with the manufacturer-recommended maximum stirring speed for each mixer (Table 1) and the worst-case, or rapid, medium addition method. The results of the testing in the 50 L and 200 L HyPerforma S.U.M.s and the 2,000 L imPULSE S.U.M. demonstrated T95 mixing times within 2 minutes or less, as shown in Table 4 and Figures 5–7. However, after 70 minutes of mixing at 5:1 volume in the 2,000 L HyPerforma S.U.M., the medium was not fully entrained and excessive foaming occurred. The medium addition method and maximum recommended agitation rate were possible factors contributing to the poor mixing observed at 5:1 working volume. The methods were then revised to evaluate whether low-volume hydration could be improved in the 2,000 L HyPerforma S.U.M. with more gradual medium addition and slower agitation. Another test was performed in the 2,000 L HyPerforma S.U.M. with the medium added slowly throughout the first 15 minutes of mixing and the agitation rate lowered from the manufacturer-recommended maximum stirring speed of 170 rpm (117 W/m^3) to 110 rpm (32 W/m^3), or ~27% of the maximum power. These method changes resulted in improved entrainment of the medium without foaming, and the medium reached T95 mixing times for all measured analytics in under 15 minutes, as shown in Table 4 and Figures 5–7.

Table 4. T95 mixing times and end-of-mixing pH data for all mixers tested in the 5:1 working volume rapid hydration study.

	T95 mixing time (min)			End mix
Mixer	Glucose	Osmolality	Conductivity	pH
50 L HyPerforma S.U.M.	1.0	1.0	1.5	7.31
200 L HyPerforma S.U.M.	2.0	2.0	1.0	7.50
2,000 L HyPerforma S.U.M.	13	14	13.5	7.11
2,000 L imPULSE S.U.M.	2.0	2.0	2.0	7.29

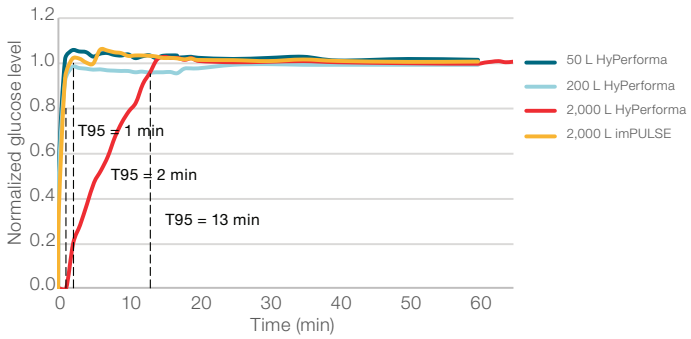

Figure 5. Normalized glucose levels from the 5:1 working volume rapid hydration study.

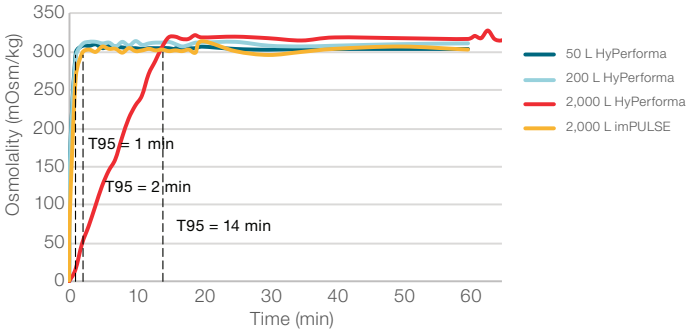

Figure 6. Osmolality from the 5:1 working volume rapid hydration study.

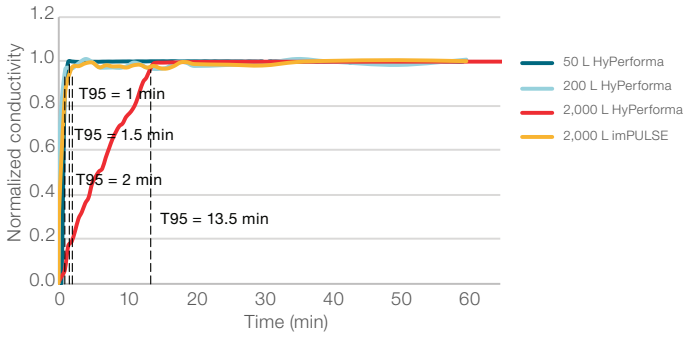

Figure 7. Normalized conductivity from the 5:1 working volume rapid hydration study.

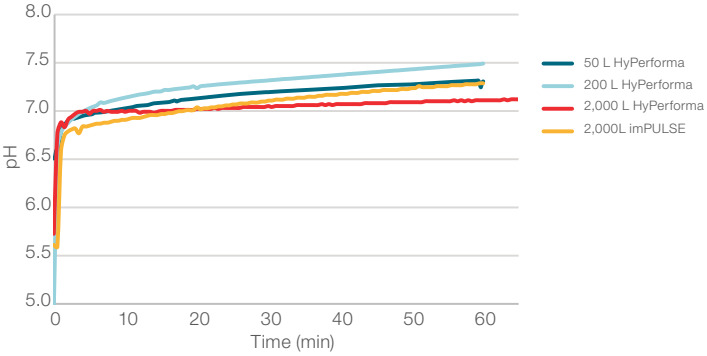

Figure 8. pH results from the 5:1 working volume rapid hydration study.

End-of-run pH was shown to be a significant factor in these mixing tests, with pH ranging from 7.3 to 7.5 after 70 minutes of mixing for the rapid addition method with maximum agitation. The modified test performed in the 2,000 L HyPerforma S.U.M. with lower mixing speed and gradual medium addition resulted in a final pH of 7.1 (Table 4 and Figure 8).

Rapid hydration study: 10:1 working volume

The results of the rapid hydration study of the 50 L HyPerforma, 200 L HyPerforma, and 2,000 L imPULSE S.U.M.s at 10:1 volume at the manufacturer-recommended maximum stirring speed with rapid medium addition demonstrated T95 mixing times of 1 to 3 minutes and end-of-mixing pH results ranging from 7.7 to 8.2. (Table 5, Figures 9–12). The 2,000 L HyPerforma S.U.M. was not tested, based on the initial poor results observed at 5:1 working volume. At 10:1 volume, higher levels of pH drift were observed compared to the drift seen with the rapid hydration method at 5:1 volume. An additional pH shift mitigation study was conducted to determine a mitigation strategy to address the pH drift issue present when mixing at low working volumes.

Table 5. T95 mixing times and end-of-mixing pH data for all mixers tested using rapid hydration at 10:1 working volume.

	T95 mixing time (min)			End mix
Mixer	**Glucose**	**Osmolality**	**Conductivity**	**pH**
50 L HyPerforma S.U.M.	2.0	2.0	1.5	7.86
200 L HyPerforma S.U.M.	2.0	2.0	–*	8.16
2,000 L imPULSE S.U.M.	1.0	3.0	3.0	7.69

* Conductivity data not gathered, due to an error that occurred with the probe during the study.

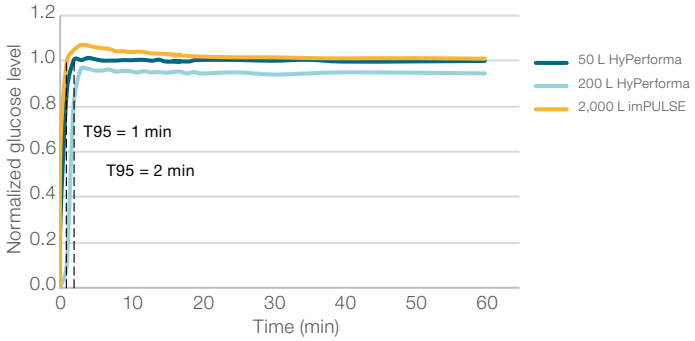

Figure 9. Normalized glucose levels from the 10:1 working volume rapid hydration study.

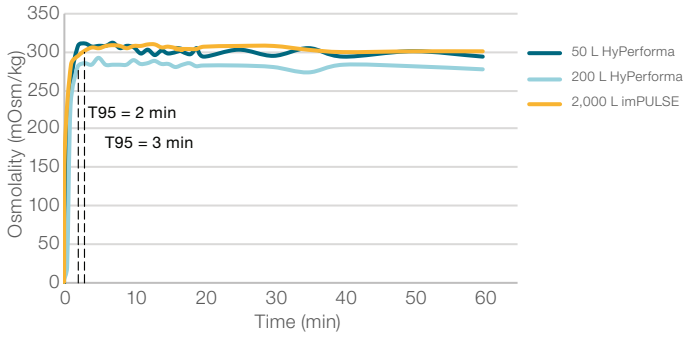

Figure 10. Osmolality from the 10:1 working volume rapid hydration study.

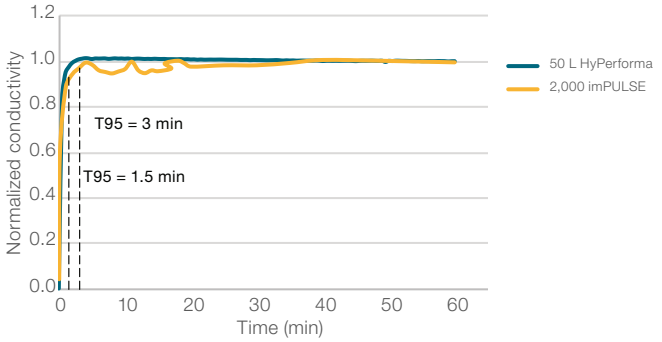

Figure 11. Normalized conductivity from the 10:1 working volume rapid hydration study.

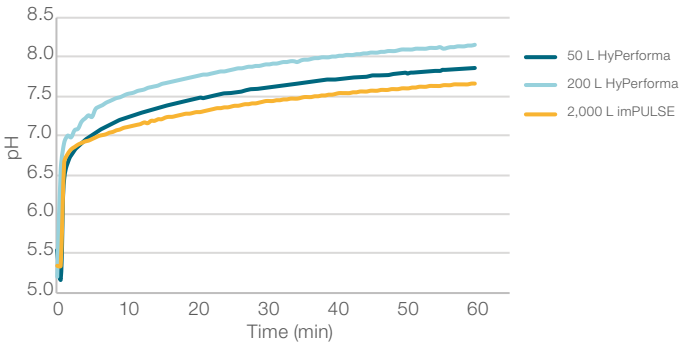

Figure 12. pH results from the 10:1 working volume rapid hydration study.

pH shift mitigation study

A rise in pH levels was observed over time when AGT medium was hydrated at a reduced 5:1 or 10:1 working volume at the manufacturer-recommended maximum stirring speed. The pH shift mitigation study was conducted with the 200 L HyPerforma S.U.M. at 5:1 working volume to evaluate whether the pH drift could be mitigated with a reduction in the agitation rate to 26%, or 52% of the maximum power (Table 1), with no modification to the medium addition method. The results of this study demonstrated that both lower agitation rates tested produced the same mixing times as what was observed in the rapid hydration study (Table 6). In addition a decrease in pH drift was observed, from pH 7.5 at the end of 70 minutes of mixing with the highest recommended maximum agitation rate (rapid hydration study), to pH 7.34 and 7.32 at 200 W/m^3 and 100 W/m^3, respectively (Table 6 and Figure 13). Some pH drift still occurred at the lower power inputs; however, the increase in pH was considerably less and allowed the medium to stay within the expected pH range.

Table 6. The T95 mixing times and end-of-mixing pH data for the 200 L HyPerforma S.U.M. using maximum and reduced agitation rates at 5:1 working volume.

		T95 mixing time (min)			
Agitation	Percent of maximum power	Glucose	Osmolality	Conductivity	End mix pH
382 W/m³ (170 rpm)	100	2.0	2.0	1.0	7.50
200 W/m³ (137 rpm)	52	2.0	2.0	–*	7.34
100 W/m³ (109 rpm)	26	2.0	2.0	1.0	7.32

* Conductivity data not gathered, due to an error that occurred with the probe during the study.

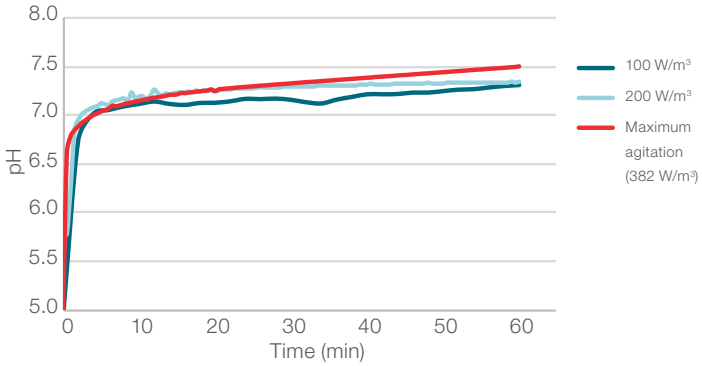

Figure 13. pH recorded during the pH shift mitigation study for the 100 W/m³, 200 W/m³, and 382 W/m³ (maximum) settings.

Figures 14 and 15 show the glucose and osmolality data obtained during the pH shift mitigation study. Despite the two significant power decreases, the T95 mixing time of 2 minutes was achieved, similar to those demonstrated at the maximum recommended power for all analytics tested (Table 6).

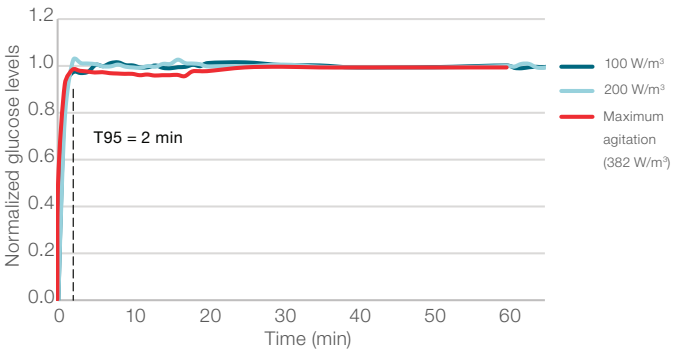

Figure 14. Normalized glucose levels from the pH shift mitigation study.

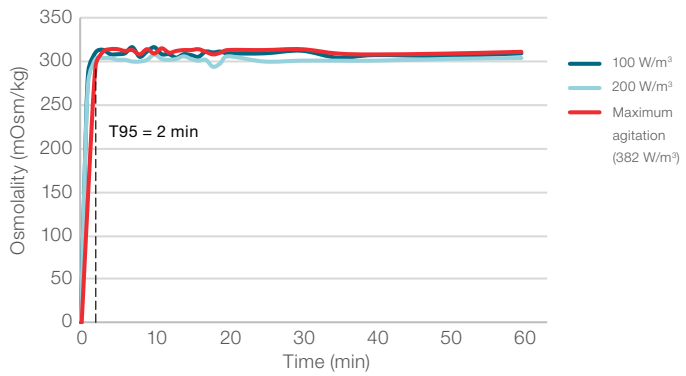

Figure 15. Osmolality levels from the pH shift mitigation study.

Conclusions

Overall, in the full-volume rapid hydration study, with rapid AGT medium addition and the maximum recommended agitation, homogeneity occurred more quickly at smaller scale in the 50 L and 200 L HyPerforma S.U.M.s (2 and 3 minutes) compared to the two larger 2,000 L HyPerforma and imPULSE S.U.M.s (45.5 and 30 minutes). Homogeneity was determined based on the medium reaching a T95 mixing time (or 95% of stable final value) for conductivity, glucose levels, and osmolality. At lower working volumes of 5:1 or 10:1, the T95 mixing times for AGT medium were rapidly achieved within 3 minutes in the 50 L HyPerforma S.U.M., 200 L HyPerforma S.U.M, and 2,000 L imPULSE S.U.M. However, effective mixing did not initially occur at 5:1 working volume in the 2,000 L HyPerforma S.U.M. The medium did not fully entrain into solution after 70 minutes of mixing, due to formation of a medium island on the fluid surface along with excessive foaming. The medium was added as quickly as possible during this testing with the manufacturer-recommended maximum stirring speed. In theory, this method of medium addition with aggressive agitation, in conjunction with the wider geometry of the 2,000 L HyPerforma S.U.M. (larger diameter-to-height ratio than the other HyPerforma S.U.M.s, and reduced overall liquid proximity to the impeller), were likely contributing factors.

Additional testing in the 2,000 L HyPerforma S.U.M. at the 5:1 volume with slower medium addition and agitation rates was successful with observed T95 mixing times under 15 minutes. The medium was easily in suspension without island formation or excessive foaming with slower agitation (~27% of maximum power) and slower medium addition (15-minute time frame). These results show that AGT medium can be easily and rapidly hydrated in the 2,000 L HyPerforma S.U.M. at lower working volumes through proper process development without aggressive agitation, manual intervention, or long mixing times being necessary to ensure complete homogeneity.

During mixing at low working volumes with the manufacturer-recommended maximum stirring speed, there was a distinct upward shift in pH in all vessels tested; the final medium pH drifted into higher ranges of 7.7 to 8.2. This shift was most likely the result of CO_2 degassing from the medium because of the increased gas/liquid surface area associated with the lower working volume as well as aggressive agitation that increased the transfer rate and directly entrained additional gas into the medium to further accelerate stripping. The degassing occurs because of the presence of sodium bicarbonate in the AGT medium formulation. The pH shift could be corrected with the addition of acid or base; however, studies have shown that acid and base addition can be correlated with process variability and osmolality increase [4,5]. Generally, it is a best practice to avoid addition of acid or base to AGT media or other cell culture media containing sodium bicarbonate.

As shown in the pH shift mitigation study, the issue of pH drift was successfully corrected by decreasing the agitation rate. When the lower agitation rates of 26% (100 W/m^3) and 52% (200 W/m^3) of the manufacturer-recommended maximum stirring speed (382 W/m^3) were evaluated in the 200 L HyPerforma S.U.M. at 5:1 volume (40 L), both reduced agitation rates demonstrated comparable 2-minute T95 mixing times and end-of-mix pH reduced from 7.5 to 7.3. In conclusion, decreasing the power input did not increase the mixing time necessary to achieve homogeneous mixing, and pH drift was easily mitigated. This study also supports that hydration time for AGT medium preparation can be shortened when optimized operating settings for S.U.M. equipment are utilized. Furthermore, media consistency and facility capacity are likely to be improved overall. Reducing processing time can not only reduce labor time but also improve overall quality by reducing risks associated with bioburden, capacity reduction, and pH shift.

The HyPerforma and imPULSE S.U.M.s were demonstrated to perform rapid homogeneous media hydration across a variety of scales and working volumes with proper operating conditions. In today's competitive, time-constrained bioprocessing environment, when these S.U.M.s are coupled with AGT dry-format media the industry's need for rapid medium hydration can be met, while avoiding pH drift and potential cell culture performance inconsistency.

Recommendations

HyPerforma S.U.M.s

When working at full volume in the smaller 50 L and 200 L vessels, AGT medium hydration was easily achieved with rapid media addition; however, as a best practice we recommend (at all mixer scales and working volumes) incrementally adding smaller amounts of AGT medium over time to avoid formation of a medium island. At full volume the manufacturer-recommended maximum stirring speed can be used without major concern, but extended mixing times should be avoided to minimize pH drift. When working at low working volumes, we recommend using a lower agitation rate. In addition, because of the geometry of the 2,000 L HyPerforma S.U.M., with its larger diameter-to-height ratio, we do not recommend mixing AGT media at lower than 5:1 working volume.

During mixing at all scales and especially at low working volumes, we recommend monitoring pH throughout the mixing process, and if pH drift occurs, reduce the agitation rate and overall mixing time to help mitigate drift. Our studies indicated that when these method modifications were implemented, medium island formation and foaming were avoided while medium homogeneity still occurred in a reasonable time frame without excessive pH drift.

We recommend that medium homogeneity is best determined by reaching two or more objective criteria, such as T95 mixing times, and not relying only on visual measures of medium entrainment or dissolution. Conductivity and pH can be easily recorded online using the Touchscreen Console, while osmolality and glucose are typically easy and quick to measure offline. We do not recommend using pH to determine homogeneity, as it reaches a steady state much faster than the other analytics.

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When a new medium or feed is implemented into a process, we recommend initially performing similar mixing testing at expected operation volumes, to determine the length of time and ideal agitation rate required to reach a stable value for the desired analytics. This is essential since other media and feeds may not hydrate in the same manner as the AGT medium tested in these studies. A small amount of time spent on process development determining the hydration method can effectively reduce extended mix times and excessive pH drift, as well as inconsistent hydration and medium performance. To further simplify the hydration process, the Touchscreen Console can be used to create a customizable recipe that sets the mixer to run at the determined hydration agitation rate and time and then shift to a lower speed after hydration to allow consistent homogeneity without pH drift. Notifications can also be sent to alert that the batch is ready to be filled to final volume and final filtered.

2,000 L imPULSE S.U.M.

The same best practice recommendations for AGT medium hydration outlined with the HyPerforma S.U.M.s also apply to the 2,000 L imPULSE S.U.M. In addition, we recommend adding a liquid recirculation loop to this mixer running at ~10 L/min via a peristaltic pump if the process will be mixing above 50% working volume. Due to the unique design of the imPULSE S.U.M., low–working volume mixing can be easily and rapidly achieved down to 10:1 working volume. However, if the mixer head is to be operated for one hour, we recommend filling the system to 20% or more and run the mixer for at least 10 prior minutes to ensure the rolling diaphragm is properly seated and taking time to adjust as needed before mixing below 20%. Smaller-size imPULSE S.U.M.s are available if a smaller mixer is desired for a specific application.

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