Mixing efficiencies for the 2,000 L HyPerforma Single-Use Mixer

Introduction

The Thermo Scientific™ 2,000 L HyPerforma™ Single-Use Mixer (S.U.M.) provides rapid mixing for large-scale upstream and downstream processes. The addition of the Touchscreen Console to the 2,000 L HyPerforma S.U.M. provides real-time sensor visualization, data logging, and the ability to automate user-defined recipe mixing. To demonstrate the superior mixing capabilities for various applications and to determine optimal mixing parameters, five different mixing studies (Table 1) were performed with the 2,000 L HyPerforma S.U.M. at various working volumes and mixing speeds. The materials used in the mixing studies were chosen to demonstrate the way buffers (NaCl solution), complex media (Gibco™ AGT™ medium), and viscous liquids (corn syrup) mixed in the 2,000 L HyPerforma S.U.M.

Along with the mixing studies, a study was performed to demonstrate the ability of the 2,000 L HyPerforma S.U.M. to hydrate media supplements. In some cell culture applications, it is advantageous to use sucrose or other oligosaccharides as a media supplement. These feeds are sometimes used for optimizing protein glycosylation, which can improve therapeutic recombinant protein efficacy. Perhaps more common is the need to prepare sucrose at 10–35% concentrations to provide cushion in downstream post-lysate protein purification to improve efficacy. Sucrose can be quickly loaded, settles rapidly, and has an inherently long hydration time when prepared at ambient temperature. Due to these properties, hydration of concentrated sucrose granules is a challenging worst-case application for an S.U.M. Two mixing studies were performed, with a 10% sucrose solution at full volume and a 20% sucrose solution at 2:1 working volume. These studies were attempted with the goal of completing setup and hydration operations in 2 hours to demonstrate that a customer could easily achieve a full feed formulation process consistently and easily within a standard 8-hour work shift.

Materials and methods

A standard 2,000 L HyPerforma S.U.M. was used with a standard 2,000 L Thermo Scientific™ HyPerforma™ S.U.M. BioProcess Container (BPC) modified with sampling ports and probe ports at the top, middle, and bottom of the BPC.

Table 1. Mixing studies performed on the 2,000 L HyPerforma S.U.M.

Mixing type	Mixing material	Concentration	Analytics
Liquid–liquid	NaCl solution	350 g/L NaCl in deionized (DI) H_2O	Conductivity
Liquid–solid	NaCl granules	1 M (final)	Conductivity, osmolality
Liquid–solid	AGT medium (granules)	1X (final)	Conductivity, osmolality, pH, glucose concentration, visual
Liquid–solid	Sucrose	10% and 20% solutions (final)	Osmolality, visual
Viscous liquid–liquid	Corn syrup with NaCl solution	80–88% corn syrup	Conductivity

To test a wide range of applications for the 2,000 L HyPerforma S.U.M., mixing studies were performed at various working volumes. The manufacturer-recommended maximum stirring speed, which was the point where maximum agitation occurs without excessive shaft wobbling or impeller splashing, was determined through visual inspection (Table 2). The corresponding power was determined using Equation 1 [1] with a power number equal to 2.1 and the maximum stirring speed observed.

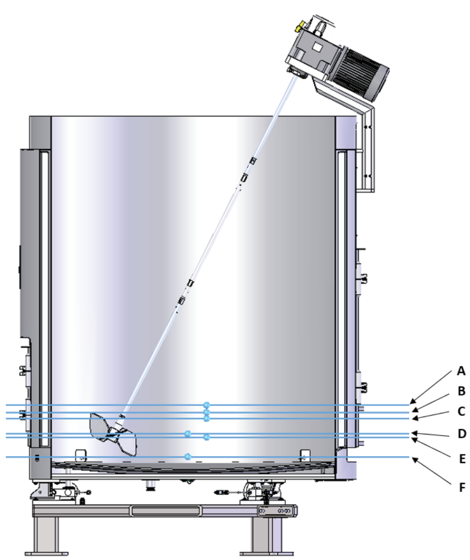
$$P = N_p \rho N_i^3 D_i^5 \qquad \text{(Equation 1)}$$

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

Table 2. The manufacturer-recommended maximum stirring speeds determined for working volumes of 10:1, 5:1, 2:1, and full volume.

Working volume	Manufacturer-recommended maximum stirring speed
10:1 (200 L)	128 rpm (100 W/m^3)
5:1 (400 L)	170 rpm (117 W/m^3)
2:1 (1,000 L)	350 rpm (407 W/m^3)
Full volume (2,000 L)	350 rpm (204 W/m^3)

The impeller coverage at various locations on the 2,000 L HyPerforma S.U.M. was determined through visual inspection, and the results are given in Figure 1.



	Description	Height	Volume (L)
A	20% working volume	29.8 cm (11.75 in.)	400
B	Top of impeller	26.7 cm (10.5 in.)	350
C	Probe belt covered	24.8 cm (9.75 in.)	324
D	10% working volume	16.5 cm (6.5 in.)	200
E	Middle of impeller	14.6 cm (5.75 in.)	186
F	Bottom of impeller	8.3 cm (3.25 in.)	66

Figure 1. Impeller coverage at various locations on the 2,000 L HyPerforma S.U.M. and the corresponding heights and volumes. Height was measured as the distance from the bottom center of the vessel to the top of the fluid at a specific volume or location.

Liquid–liquid mixing: NaCl solution

Conductivity probes were inserted into the bottom, middle, and top ports of the BPC. The BPC was filled with deionized water to four separate working volumes: 10:1, 5:1, 2:1, and full volume. At each volume, the following power inputs were tested in triplicate: minimum (30 rpm), 20 W/m^3, 100 W/m^3, and maximum (350 rpm). Although the manufacturer-recommended maximum stirring speed was greater than 100 W/m^3 at 5:1 working volume, only the 100 W/m^3 power input was tested to produce more comparative data between working volumes.

The predetermined agitation rate was set on the 2,000 L HyPerforma S.U.M., and mixing was allowed for 2 minutes so that flow patterns could stabilize. A small volume of concentrated NaCl solution (350 g/L in DI H_2O) was pipetted into the mixer as close to the surface of the water as possible over a span of 3–4 seconds. Conductivity data were recorded for 5 minutes with a data logger. This process was repeated for each power input at each volume in triplicate. An average T95 mixing time—when the measured value reaches 95% of the final stable value—was calculated as the average for the slowest-responding sensor among the test replicates under each test condition.

Liquid–solid mixing: NaCl granules

Conductivity probes were inserted into the bottom, middle, and top ports of the BPC. The BPC was filled with DI water to 5:1 and full working volumes. For each working volume, the manufacturer-recommended maximum stirring speed was determined and used (Table 2). The agitation rate was set, and enough NaCl was added to the mixer to make a 1 M NaCl solution. Samples were taken every minute for the first 20 minutes, every 5 minutes for the next 20 minutes, every 10 minutes for the next 20 minutes, and every minute for the last 10 minutes. Samples of 10 mL were taken from the bottom, middle, and top ports for the full-volume test and from the bottom sampling port for the 5:1 working volume. Conductivity was measured online, and osmolality was tested and recorded after samples were collected.

Liquid–solid mixing: AGT medium

A conductivity probe and a pH probe were inserted into the BPC through the top and bottom sensor ports. The BPC was filled with DI water to 90% of the 5:1 and full working volumes. At each working volume, the manufacturer-recommended maximum stirring speed was used (Table 2).

The mixer was turned on to the predetermined agitation rate, and the manufacturer-recommended amount of AGT medium was quickly added to the top of the mixer. This method of adding medium was changed during the study, as explained in the Results section. Samples were taken over a 70 minute span using the same sampling method described in the previous section. Samples of 10 mL were taken from the bottom, middle, and top ports for full-volume testing, and only the bottom port for the 5:1 working volume. The pH and conductivity were measured online with the Touchscreen Console. The osmolality and glucose concentrations of the samples were tested and recorded after they were collected.

Liquid–solid mixing: sucrose

Initially a mixing study was performed with a 10% sucrose solution at full volume. The BPC was initially filled with 1,400 L of DI water at ambient temperature (16.8°C), and the agitation rate was set to 350 rpm. A timer was started, and 200 kg of sucrose (food-grade sucrose was sourced for this study) was added through the addition port on the top of the BPC as rapidly as possible. After addition was complete, the BPC was filled with an additional 400 L of DI water for a final volume of 2,000 L. The S.U.M. was allowed to mix until all sucrose was determined visually to be dissolved. Osmolality samples were taken for 80 minutes from the bottom sampling port after the BPC was filled to final volume—every 2 minutes for the first 20 minutes, and every 10 minutes for the subsequent 60 minutes of mixing.

For the second sucrose mixing study, a test was performed with a 20% sucrose solution at 2:1 working volume. The BPC was initially filled with 700 L of DI water at ambient temperature (15.4°C), and the agitation rate was set to 350 rpm. A timer was started, and 200 kg of sucrose was added through the addition port on the top of the BPC. After addition was complete, the BPC was filled with an additional 100 L of DI water for a final volume of 1,000 L. The S.U.M. was allowed to mix until all sucrose was determined visually to be dissolved. During the 20% sucrose hydration study, osmolality samples were taken right after the sucrose was added and for 20 minutes before filling to the final volume (1,000 L). This better captured the curve during mixing of the sucrose powder. Samples were taken over a 110 minute span from the bottom sampling port—every minute for the first 20 minutes of mixing, and every 10 minutes for the subsequent 90 minutes of mixing.

Viscous liquid–liquid mixing: corn syrup with NaCl solution

Conductivity probes were inserted into the top, middle, and bottom of the BPC. The BPC was filled initially with 2,000 L of 88% corn syrup/12% DI water (v/v). A 350 g/L NaCl solution was made, and a 5 L mixture containing 12% of the salt solution and 88% corn syrup (v/v) was prepared. The agitation rate was set to 350 rpm, and the mixture of salt solution and corn syrup was quickly added. The viscosity of the 88% corn syrup solution in the HyPerforma S.U.M. was measured with a viscometer. The change in conductivity was monitored for 15–30 minutes, and a T95 mixing time was calculated as described in the liquid–liquid mixing section. This process was repeated in triplicate with 84% corn syrup and 80% corn syrup by draining a predetermined volume of the corn syrup solution and filling with DI water up to full volume until the desired percentage of corn syrup was achieved.

Results

Liquid–liquid mixing: NaCl solution

For liquid–liquid mixing of a salt solution, T95 mixing times were less than 4 minutes regardless of the working volume or agitation, as shown in Figure 2. At most working volumes tested, as power increased the mixing times decreased. However, there was not a significant decrease in already rapid T95 mixing times when the power input was increased above 100 W/m^3. Above 100 W/m^3, the T95 mixing times were under one minute in all cases.

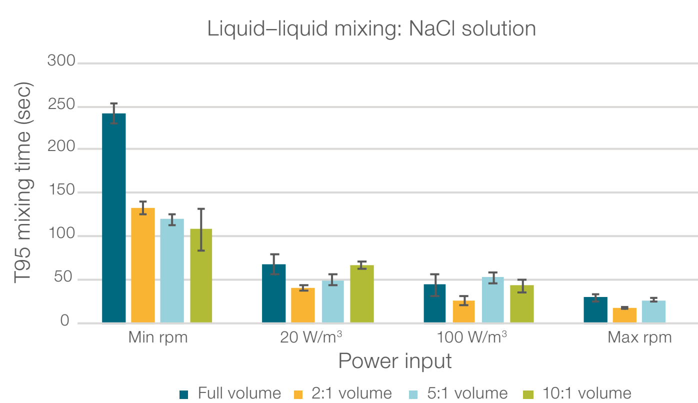


Figure 2. The average T95 mixing times (±1 standard deviation) calculated for various working volumes at minimum rpm (30 rpm), 20 W/m^3, 100 W/m^3, and maximum rpm (350 rpm).

Liquid–solid mixing: NaCl granules

For liquid–solid mixing using salt granules, the conductivity was measured for two working volumes as shown in Figure 3. The T95 mixing time was 3.5 minutes at full volume. A T95 mixing time was not determined for the 5:1 volume with conductivity or osmolality values because a steady state was never fully achieved. It was observed during this mixing study at 5:1 working volume that some of the salt granules sat at the bottom of the mixer. This produced a result where no T95 mixing time occurred. Similar results have been observed at low working volumes in the 50 L and 200 L HyPerforma S.U.M.s and the 2,000 L Thermo Scientific™ imPULSE™ S.U.M. Figure 4 shows the osmolality recorded for the tested working volumes. A T95 mixing time was reached in 2 minutes at full volume.

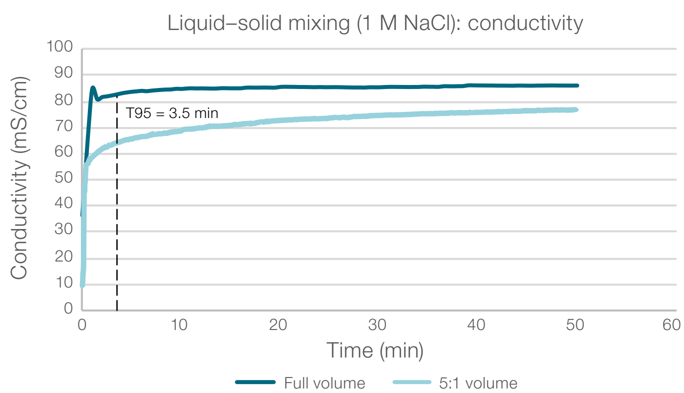


Figure 3. Conductivity recorded at 5:1 working volume and full volume.

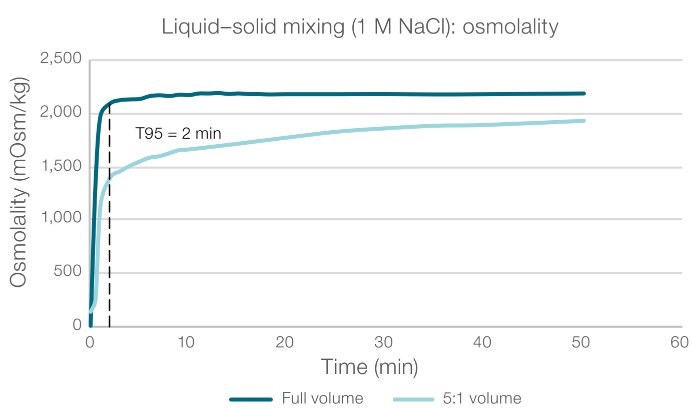


Figure 4. Osmolality recorded at 5:1 working volume and full volume.

Liquid–solid mixing: AGT medium

For liquid–solid mixing of representative AGT medium granules, the method of adding the AGT medium granules as quickly as possible (worst-case scenario) was successful at full volume (complete entrainment occurred and a T95 mixing time was reached) but was initially unsuccessful at 5:1 working volume. Excessive foaming was present, and the granules were not fully in suspension after 70 minutes of mixing the medium. The medium addition method for mixing at 5:1 working volume in the 2,000 L HyPerforma S.U.M. was revised under the premise that the initial agitation and medium addition strategies were too aggressive. Another test was performed where the medium was added slowly during the first 15 minutes of mixing, and agitation was lowered from the manufacturer-recommended maximum stirring speed at 5:1 working volume of 170 rpm (117 W/m^3), down to 110 rpm (32 W/m^3). The glucose concentration and osmolality were measured offline, and the data are reported in Figure 5. At 5:1 volume with rapid medium addition, the T95 mixing times were 25 minutes for glucose concentration and 35 minutes for osmolality. At 5:1 volume with 15-minute medium addition, the T95 mixing time was 13 minutes for glucose concentration and 14 minutes for osmolality. At full volume, the T95 mixing time was 45 minutes for glucose concentration and 35 minutes for osmolality.

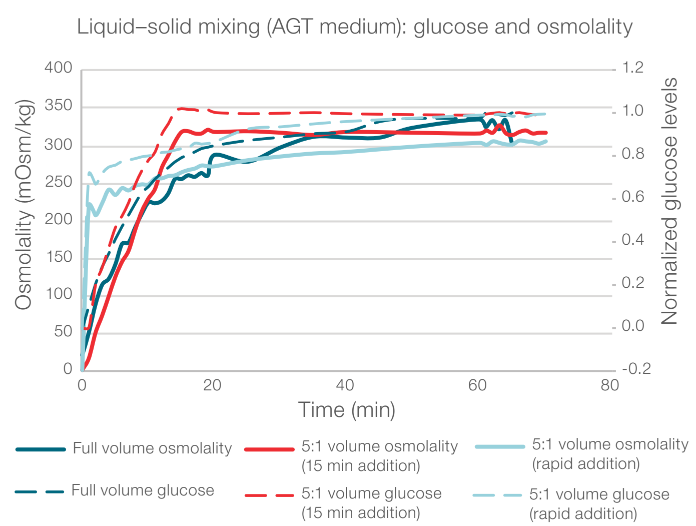


Figure 5. Glucose levels and osmolality with both methods of medium addition at 5:1 volume, and at full volume.

Similar mixing trends were observed in the conductivity data (Figure 6). At 5:1 volume with rapid medium addition the T95 mixing time was 40 minutes, and with slow addition the T95 mixing time was 13.5 minutes. At full volume the T95 mixing time was 44.5 minutes. Figure 7 shows the pH data collected during the mixing studies. An upward drift in pH occurred during the 5:1 volume mixing study with rapid medium addition and maximum agitation. It is likely that this shift is a result of degassing of CO_2 due to the low working volume and rapid mixing. This shift is not ideal, because cell metabolism can be altered by pH changes [2]. However, when 5:1 volume mixing was tested with a 15-minute medium addition time and a lower rpm, the drifting was minimized to a more acceptable level.

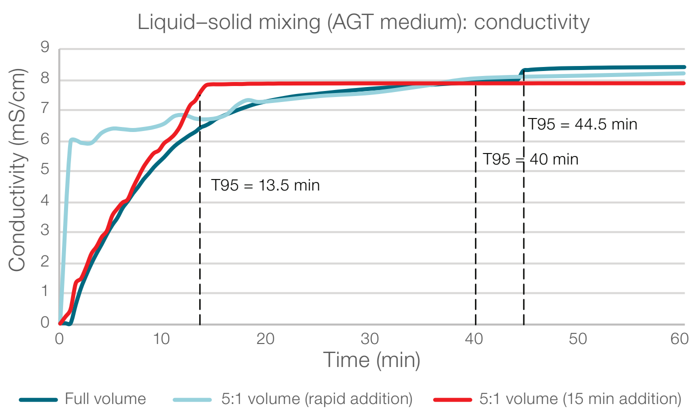


Figure 6. Conductivity with both methods of medium addition at 5:1 volume and full volume.

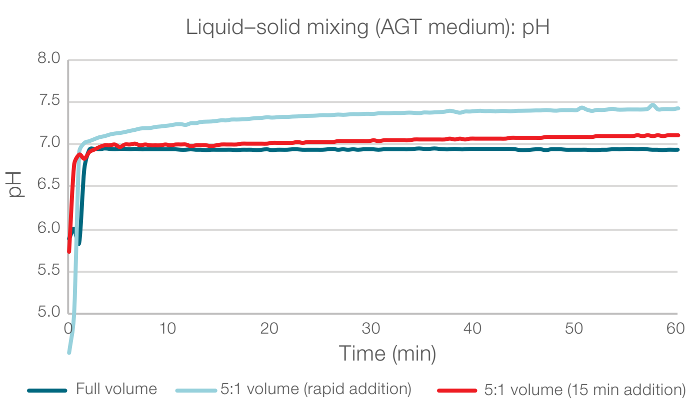


Figure 7. pH with both methods of medium addition at 5:1 volume and full volume.

Figure 8 shows photos taken at various times during the 5:1 volume mixing study in which the medium was added rapidly with maximum agitation. Some granules of the medium were present throughout the entire mixing study and excessive foaming was observed. This correlates to the data in Figures 5 and 6. An initial jump in osmolality, glucose level, and conductivity was measured, and then a steady increase was observed for the remainder of the mixing study.

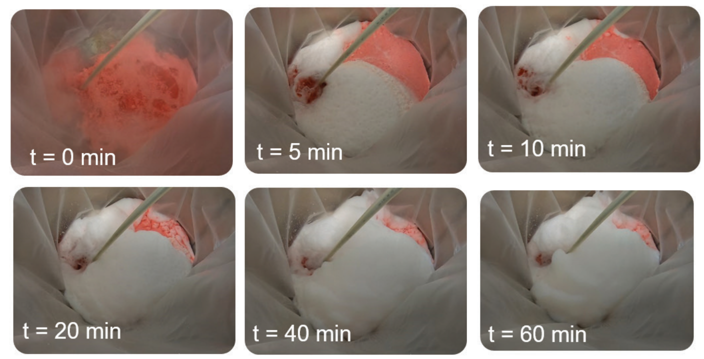


Figure 8. Photos of the fluid at the top of the mixer at various time points during the 5:1 working volume mixing study with rapid medium addition and maximum agitation.

Figure 9 shows photos taken at various times during the 5:1 volume mixing study in which the medium was added over a 15-minute span with a lower agitation rate. After 15 minutes of mixing, all the medium granules had been added. After 28 minutes of mixing, all the powder was completely in suspension and foaming was diminished.

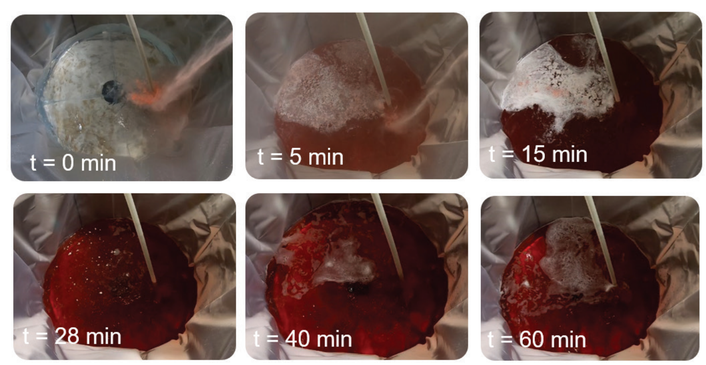


Figure 9. Photos of the fluid at the top of the mixer at various time points during the 5:1 working volume mixing study with 15-minute medium addition and slower agitation.

Figure 10 shows photos taken at various times during the full-volume test. After 20 minutes of mixing, the powder was completely in suspension and foaming was diminished. It is important to consider visual observation of entrained medium components when mixing as well as the dissolution of those components for a fully mixed solution. Process qualification must take place through measuring analytics, such as glucose levels, osmolality, and conductivity. This will help to ensure complete solution homogeneity without overmixing to avoid pH shifts described previously.

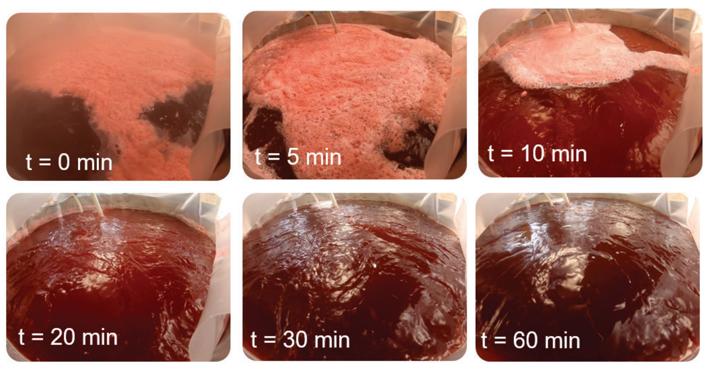


Figure 10. Photos taken of the fluid at the top of the mixer at various time points during the full volume AGT medium mixing study.

Liquid–solid mixing: sucrose

During the 10% sucrose hydration at full volume, the sucrose was added over 7 minutes, and the final temperature of the solution was 16.8°C. The total mixing time, including powder addition and time to fill to quantity sufficient (QS), was 2 hours and 19 minutes. The osmolality data gathered after the tank was filled to QS are shown in Figure 11. The data suggest that despite the 2 hours and 19 minutes total of mixing time, 95% of the sucrose was incorporated during the first 40 minutes of mixing. Homogeneity (assessed visually) correlated with a mixing time of under 40 minutes as well. This suggests a T95 mixing time of under 40 minutes. Figure 12 shows the sucrose solution after the sucrose was fully incorporated. The green tint was due to the purity level of the sucrose sourced for testing.

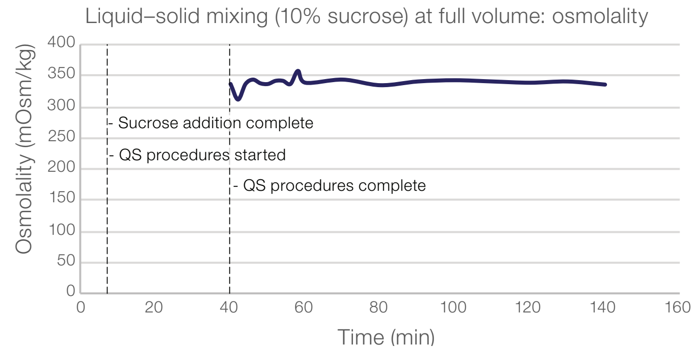


Figure 11. Osmolality recorded over 139 minutes of mixing during the 10% sucrose testing at full volume in the 2,000 L HyPerforma S.U.M.

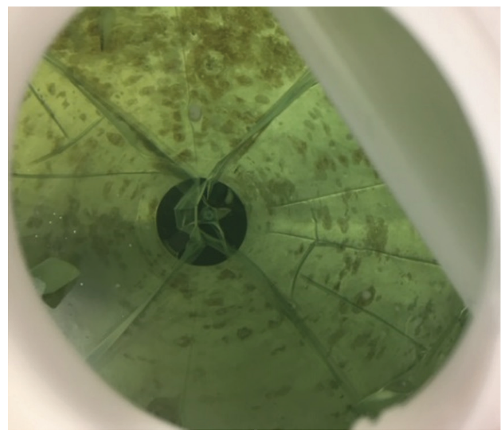


Figure 12. Photograph taken through the powder addition port after 139 minutes of mixing, when sucrose was fully solubilized during the 10% sucrose testing at full volume (2,000 L).

During the 20% sucrose hydration at 2:1 working volume, the sucrose was added over 11 minutes and the final temperature of the solution was 15.3°C. The total mixing time, including powder addition and time to fill to QS, was 2 hours. The osmolality results (Figure 13) show that despite the 2 hour total of mixing time, 95% of the sucrose was incorporated during the first 40 minutes of mixing. Homogeneity (assessed visually) correlated with a mixing time of under 40 minutes as well. This suggests a T95 mixing time of under 40 minutes.

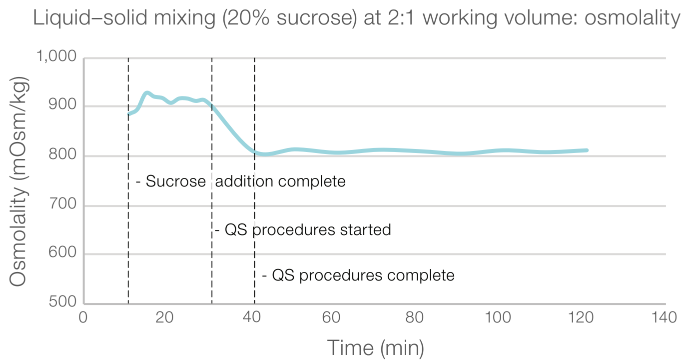


Figure 13. Osmolality recorded during 120 minutes of mixing during the 20% sucrose testing at 2:1 working volume (1,000 L) in the 2,000 L HyPerforma S.U.M.

Through visual observation, it was estimated that 95% of the sucrose was in solution after 40 minutes of mixing in both sucrose studies. With slower sucrose addition it may be possible to further decrease the total solubilization time in both studies. It is also important to note that very little settling of the sucrose occurred during either sucrose mixing study, even with the nonideal method of rapid addition of sucrose (minimal settling did occur on the vessel floor and adjacent to BPC film seams), and undissolved sucrose was kept mostly suspended through mixing.

Viscous liquid–liquid mixing: corn syrup with NaCl solution

The measured viscosity of the corn syrup mixtures containing approximately 80–88% corn syrup by volume ranged from 309 cP to 894 cP, within the ranges of medium- to high-viscosity fluids seen in some applications. The conductivity data recorded are shown in Figure 14, with longer mixing times observed as viscosity increased. The average T95 mixing times were determined to be 2.2 minutes for 80% corn syrup (316 cP), 3 minutes for 84% corn syrup (518 cP), and 8 minutes for 88% corn syrup (894 cP).

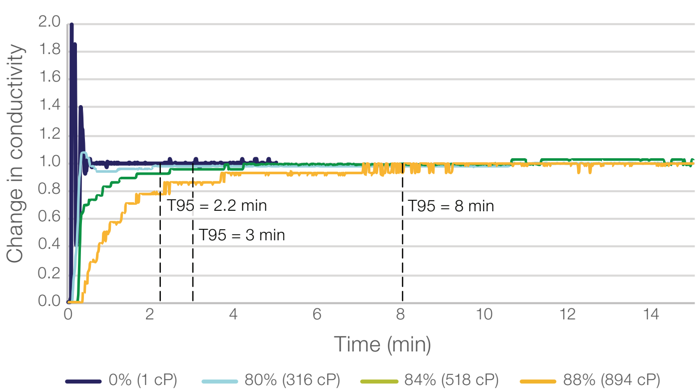


Figure 14. Conductivity data recorded during viscous liquid–liquid mixing with a solution range of 0–88% corn syrup.

Conclusions and recommendations

For liquid–liquid mixing, the 2,000 L HyPerforma S.U.M. was shown to mix all working volumes in under 4 minutes regardless of the mixing speed used. At lower working volumes, rapid mixing was generally observed. However, mixing sinking solids in the 2,000 L HyPerforma S.U.M. was found to be problematic at 5:1 working volume. Settling occurred, necessitating longer mixing times. While hydration of sinking solids at 5:1 working volume showed less efficient performance, liquid–liquid mixing was shown to be within the same times as full volume at equivalent power input. This highlights the 2,000 L S.U.M. as a solution for large-volume hydration and showcases superior mixing throughout the entire draining process and its working volume range. Hydration of AGT medium was successful at full volume and 5:1 volume. However, mixing at 5:1 working volume was more efficient when the medium was added over a 15-minute time frame and when lower agitation than the manufacturer-recommended maximum setting was used. Under the extreme conditions tested (quick addition of bulk powder), homogeneity was achieved at full volume in less than 45 minutes. With more ideal, less aggressive formulation techniques, mixing times could be lowered further for specific applications.

In both the 10% (2,000 L) and 20% (1,000 L) sucrose hydration studies, the 200 kg of sucrose was added rapidly as a worst-case scenario. We believe that adding the sucrose in standard 10–20 kg batch aliquots over a total time of 25 minutes instead of as rapidly as possible would result in a T95 mixing time of less than 30 minutes in both test cases, and minimal or no setting would occur. Even though the worst-case scenario for sucrose addition was used, the observed 40 to 60 minute hydration time was considered sufficient. This demonstrated that even some of the most challenging large-scale formulations can be completed confidently and rapidly with the 2,000 L HyPerforma S.U.M.

The 2,000 L HyPerforma S.U.M. was also found to be capable of mixing medium- to high-viscosity fluids in under 8 minutes. Table 3 shows when each solution reached a T95 mixing time or visual homogeneity based on the analytic measured.

Table 3. Average mixing times for mixing of various materials in the 2,000 L HyPerforma S.U.M.

Mixing study	Mixing material	Working volume	Agitation	Analytic	T95 mixing time (min)
Liquid–liquid	NaCl solution	200 L (10:1)	128 rpm (100 W/m^3)	Conductivity	0.7
		400 L (5:1)	350 rpm (1,018 W/m^3)	Conductivity	0.5
		1,000 L (2:1)	350 rpm (407 W/m^3)	Conductivity	0.3
		2,000 L (full volume)	350 rpm (204 W/m^3)	Conductivity	0.5
Liquid–solid	NaCl granules	400 L (5:1)	170 rpm (117 W/m^3)	Conductivity	–
				Osmolality	–
		2,000 L (full volume)	350 rpm (204 W/m^3)	Conductivity	3.5
				Osmolality	2.0
Liquid–solid	AGT medium	400 L (rapid addition)	170 rpm (117 W/m^3)	Conductivity	40
				Osmolality	35
				Glucose	25
				Visual	–
		400 L (15 min addition)	110 rpm (32 W/m^3)	Conductivity	13.5
				Osmolality	14
				Glucose	13
				Visual	28
		2,000 L (full volume)	350 rpm (204 W/m^3)	Conductivity	44.5
				Osmolality	35
				Glucose	45
				Visual	20
Liquid–solid	10% sucrose	2,000 L (full volume)	350 rpm (~211 W/m^3)	Visual	40
				Osmolality	40
Liquid–solid	20% sucrose	1,000 L (2:1 volume)	350 rpm (~440 W/m^3)	Visual	40
				Osmolality	40
Viscous liquid	High-viscosity liquid (800–1,000 cP)	2,000 L (full volume)	350 rpm (~268–272 W/m^3)	Conductivity	8.0
Viscous liquid	Medium-viscosity liquid (200–400 cP)	2,000 L (full volume)	350 rpm (~265–268 W/m^3)	Conductivity	3.0

For optimal mixing with a variety of materials, we suggest filling the mixer, setting the desired agitation, and adding product incrementally. A slow addition rate will allow solids to easily become entrained in the liquid without forming a large island on top of the fluid or large clumps. If a complex medium is being prepared at 5:1 working volume, we recommend that the mixer be set to an agitation lower than the manufacturer-recommended maximum stirring speed. Lowering the agitation, decreasing overall processing time, and adding medium incrementally help ensure that the pH remains in range throughout the hydration process. It will also help ensure that the medium is hydrated fully and easily. Due to the geometry of the 2,000 L HyPerforma S.U.M., hydrating complex media at 10:1 working volume or lower is not recommended.

The 2,000 L HyPerforma S.U.M. performs complete, rapid mixing that can be used in a variety of large-scale upstream and downstream processes that contain a wide variety of materials, ranging from liquids, floating solids, sinking solids, and viscous liquids. With the addition of the Touchscreen Console, the 2,000 L HyPerforma S.U.M. is a user-friendly, rapid, and robust S.U.M. that meets competitive mixing requirements that are essential in today's mixing industry.

References

1. Doran PM (2013) *Bioprocess Engineering Principles*, Second Edition. Elsevier: Waltham, MA.
2. Hagrot E (2011) Development of a culture system for modeling of pH effects in CHO cells. Student thesis. KTH School of Biotechnology, Department of Process Technology, Stockholm, Sweden.

Find out more at **thermofisher.com/sum**