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Viral Inactivation Efficacy Using the Flexsafe® Pro Mixer

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Abstract

The main challenge during viral inactivation with single-use mixing bag is to ensure homogeneous mixing and contact of the inactivation agent through the entire volume of the bag assembly used for this process step.

The study aim is to demonstrate that the Flexsafe® Pro Mixer is adequate for a viral inactivation step with exposure to a low-pH solution. The objective is to compare the solute concentration in "the cup" (portion of the bag assembly holding the magnet of the impeller) to the bulk contents of bag with both low-viscosity (1 cP) and high-viscosity (33 cP) solutions and determine if there is a stagnant zone or delayed mixing.

For the 1 cP solution at 80 rpm, the cup conductivity is within 97% of the bulk conductivity after three minutes of mixing.

For the 33 cP solution at 80 rpm, the conductivity in the cup is within 89% of bulk solution conductivity after 30 minutes.

For a 33 cP solution but with higher agitation speed of 100 rpm, the cup conductivity reaches 95% of bulk conductivity after 20 minutes of mixing. Higher mixer speed contributed to faster equilibration between bulk and cup conductivity.

This application study demonstrates effective mixing between the bulk material in the bag and the cup ensuring that the entire contents of the bag come into contact with the viral clearance agent. The mixing effectiveness and suitability of the Flexsafe® Pro Mixer for viral clearance is confirmed in this study by measuring a similar solute concentration in the cup and in the bulk of the single-use bag in both low-viscosity and high-viscosity solution.

For More Information, Visit
www.sartorius.com/en/products/fluid-management/mixing/flexsafe-pro-mixer

Introduction

Many biopharmaceutical manufacturers have elected to use magnetically driven, bottom-mounted mixers for their mixing processes because of the minimal risk of contamination, low-shear stress, and ease of use.

Flexsafe® Pro Mixer technology combines speed and efficiency to deliver high-performance mixing during powder dissolution and a levitating impeller to preserve the product during low-shear blending applications (1). The Flexsafe® Pro Mixer has proven its ability to dissolve buffer powders even in worst-case situations, such as high volume and high concentration (2).

Previous studies with the Flexsafe® Pro Mixer technology have demonstrated that the extent of shear exposure is unlikely to cause protein aggregation or denaturation in the biopharmaceutical processing operations (3).

The objective of this study is to demonstrate effective, homogenous mixing for viral inactivation and consistent, efficient mixing in both the bulk contents of the bag and the cup portion.

Mixing Technology

Flexsafe® Pro Mixer Bags (50–1,000 L) contain a central magnetic impeller assembly. The mixing technology principle is based on electromagnets in the motor drive interacting with a fully encapsulated permanent magnet inside the mixing bag. The impeller is inside a cup — a port welded into Flexsafe® Pro Mixer Bags — that holds the impeller in place and sits on the drive unit (Figure 1). When in use, the impeller is stabilized away from the sides and bottom of the cup via magnetic coupling with the rotor magnet. There are no moving parts within the motor and no contact with the impeller, as shown in Figure 2.

In biomanufacturing, mixing is a physical operation that reduces non-uniformities in fluid by eliminating gradients of concentration and temperature that cause non-ideal flows. Traditionally, viral inactivation takes place in stainless steel vessels, but with the introduction of single-use technology, many manufacturers have switched their processes to take advantage of its many benefits.

Single-use technology offers operational flexibility, reduced capital infrastructure costs, and increased efficiency by minimizing the time and validation requirements associated with hardware cleaning.

A recent publication evaluated low pH as a robust process for viral inactivation (4), in addition to protocols available from ASTM (5). The viral inactivation step is dedicated exclusively to viral reduction to meet the International Conference on Harmonization (ICH) Q5A guidance for viral safety (6).

A typical low-pH viral inactivation operation (Figure 3) includes an acidification step where a Protein A eluate (PAE) is adjusted to a pH of approximately 3.6 – 3.7. These mildly acidic conditions have a strong inactivating effect on many enveloped viruses, and therefore contribute significantly to virus reduction.

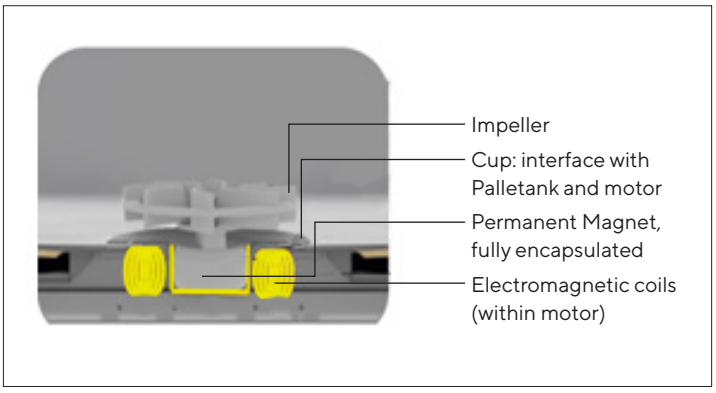

Figure 1: Mixing technology principle of the Flexsafe® Pro Mixer

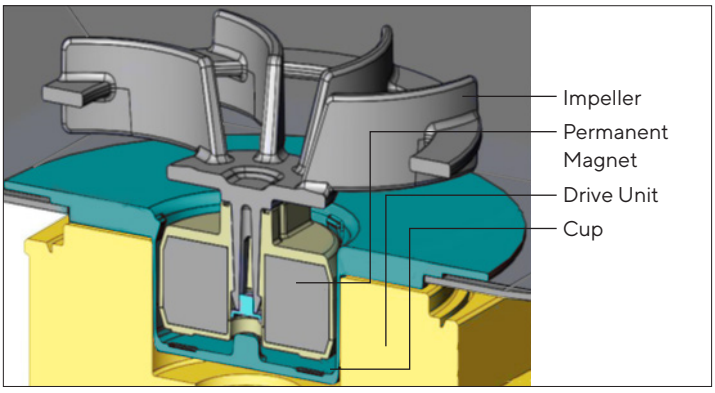

Figure 2: Section of the magnetic impeller assembly showing the impeller, the fully encapsulated permanent magnet, the cup and the drive unit

The process is very sensitive to variation: inactivation is reduced significantly at a pH greater than 3.8, and the risk of aggregate formation begins at a pH lower than 3.6.
The viral inactivation process usually takes place in a stirred tank or a single-use mixing bag in which pH, hold time, and temperature are controlled. After adjusting pH to the desired acidic value, operators incubate the pool contents for a specified duration and temperature to achieve effective virus inactivation. Afterward, the solution is neutralized and adjusted as needed for the next processing step (7).

During the inactivation process, all material within a batch must reach the target pH value for the specified duration.
If this doesn't occur, some material could carry still-active viral particles downstream (carryover), compromising the virus reduction. This carryover can occur when the same single-use equipment is used before and after inactivation where liquids remain in "dead-zone" areas of a single-use mixing bag.

"Dead zones" or "dead legs" are areas in process tubing or vessels where stagnation or channeling occurs.
These stagnant zones can trap impurities that may end up in a product, but they also interfere with uniform mixing or cause delayed mixing (9). Imperfect mixing and dead zones could impede effective virus inactivation.

To mitigate the risks of carryover contamination, biopharmaceutical manufacturers have implemented a dual single-use vessel design. Process solution pH is reduced in the first single-use mixing bag, then the solution is transferred to the second single-use mixing bag for incubation. This ensures that all material within the second single-use mixing bag has been titrated (8).

Some processes use three single-use mixing bags to ensure that the entire contents of the first mixer are inactivated, including any droplets on the mixer wall or dead zones inside tubing. The three single-use mixing bags in this approach include one for low-pH viral inactivation, one for neutralization, and one for homogenization of the filtered drug substance. Still other processes combine the two mixing steps, low-pH inactivation and neutralization, into the same single-use mixing bag to keep the process within two single-use mixing bags.

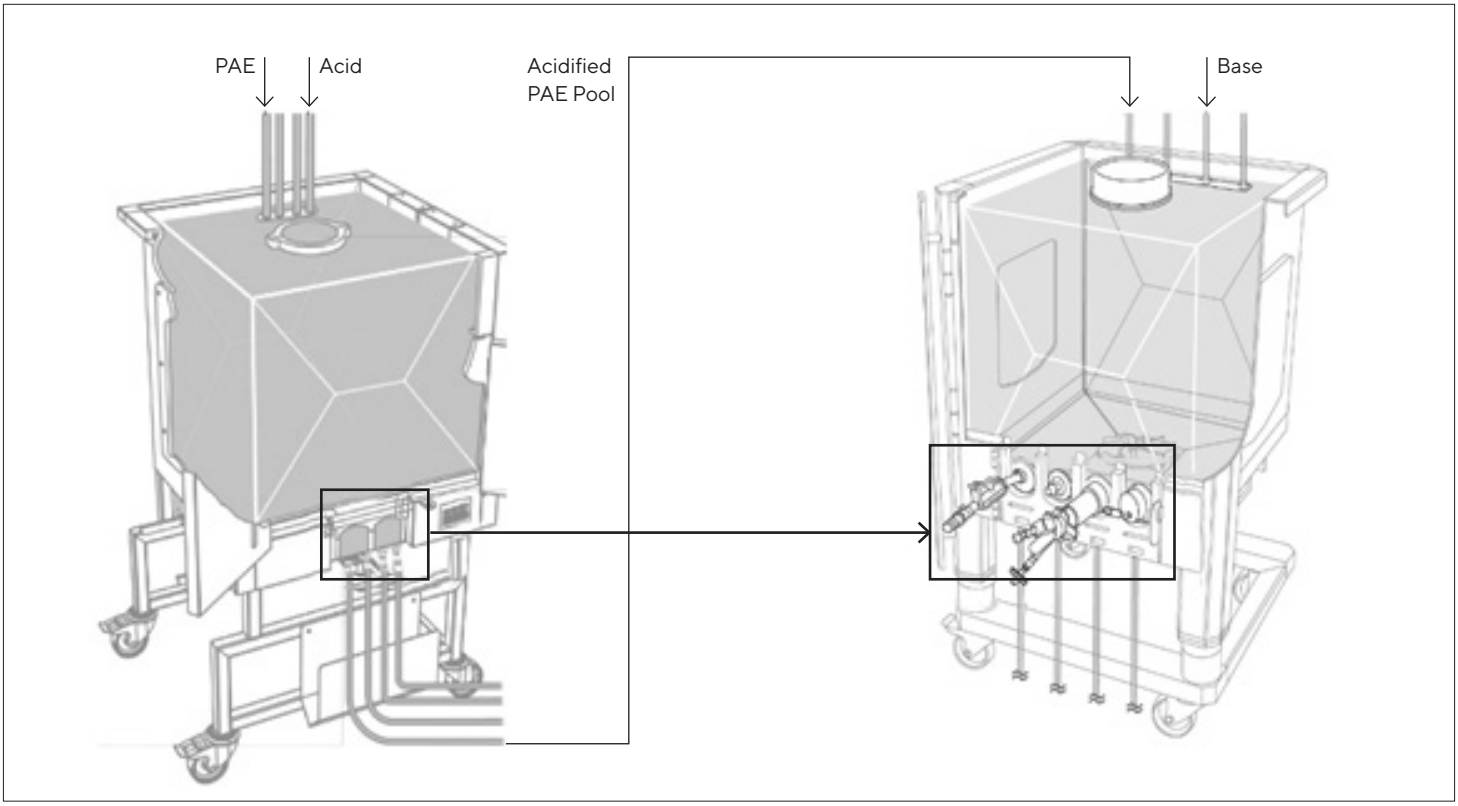

Figure 3: Low-pH viral inactivation single-use Process. Left: Acidification of a protein A eluate (PAE). Right: Neutralization of acidified PAE Pool.

Computational Evaluation of Mixing in the Cup by CFD

The computational evaluation of the Flexsafe® Pro Mixer system was performed using CFD (Computational Flow Dynamic) to understand the flow circulation in a volume of 50 L and agitation speed of 200 rpm.

The fluid was assumed to remain Newtonian, maintaining flow and a viscosity of 1 cP throughout mixing.
Simulated streamlines for the liquid inside the cup are shown in Figure 4. The volume in the cup with the magnet inserted is approximately 5 mL.

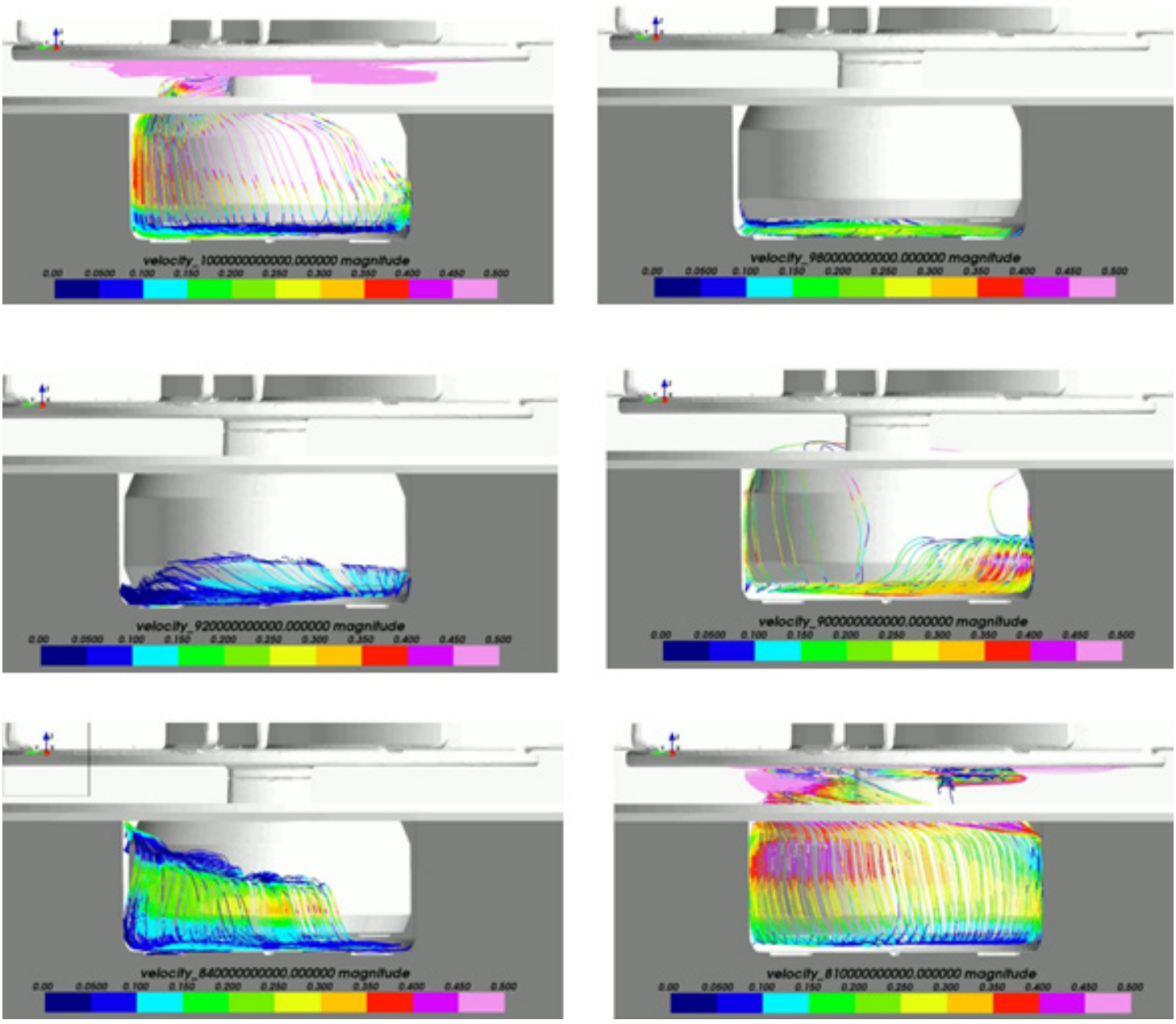

Figure 4: Velocity on streamlines below the impeller and inside the cup from the simulation of the Flexsafe® Pro Mixer of 50 L at 200 rpm.

As shown in Figure 4, after a short period of time, the material in the cup is well mixed despite its low volume and geometry. There is no dead or stagnant zone inside the cup, but fluid may have a longer residence time compared to the bulk of the Flexsafe® Pro Mixer bag. A longer pool processing time may be required to account for any differences in mixing between the cup and the bulk of the bag.

It is important to experimentally quantify the concentration gradient between the cup, the bulk, and the delay to achieve homogeneity.

Study Objective

The study aim is to demonstrate that the Flexsafe® Pro Mixer is adequate for a viral inactivation step with exposure to a low-pH solution.

The objective is to compare the solute concentration in the cup to the bulk contents of bag with both low-viscosity (1 cP) and high-viscosity (33 cP) solutions and determine if there is a stagnant zone or delayed mixing.

Materials

Consumables

- Standard 50 L Flexsafe® Pro Mixer Bags
- Deionized (DI) water (1 cP)
- Sodium chloride solution (25% wt. NaCl)
- Polyethylene glycol (PEG): a polyether compound that forms a viscous Newtonian fluid when dissolved in water (33 cP)
- Conductivity standard solution: 0.01 M KCl

Equipment

- 50 L Palletank for Mixing
- The Flexsafe® Pro Mixer Drive Unit
- Sartorius PY-C12-2S: Offline Conductivity Sensor
- Multichannel Electrochemical Meter

As shown in Figure 5, a hole was tapped into the cup to allow installation of a line to take samples.

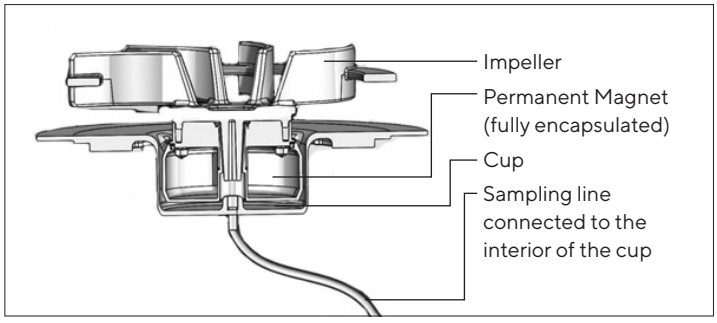

Figure 5: Section of the magnetic impeller assembly showing the impeller, the fully encapsulated permanent magnet, the cup, and its sampling line

Methods

Offline analysis of cup samples is compared with measurements from the well-mixed bulk to quantify any concentration differences and delay to achieve homogeneity.

Complete the test for both 1 cP viscosity (DI water) and 33 cP viscosity (PEG solution)

- Install the Flexsafe® Pro Mixer bag in the Palletank for Mixing.
- Fill the mixing bag with the test fluid (DI water or PEG solution) to 100 percent of its nominal volume.
- In the case of the higher viscosity test, adjust the viscosity using PEG solution to 33 cP.
- Couple the drive unit to the Palletank.
- Begin agitation (50 rpm for 1 cP, 80 rpm for 33 cP solution, and 100 rpm for 33 cp solution).
- Charge the 25 wt. percent NaCl solution tracer through the 8-inch top port of the bag, set the timer for 30 seconds and start the timer.
- After 30 seconds on the timer, flush 3 mL of holdup from the cup sampling line, then tak a 5 mL sample from the cup and a sample from the top port of the bag simultaneously.
- Measure and record the conductivity of both samples using an offline sensor.
- Before starting the next trial, flush 20 mL from the cup port to ensure the bag and cup contain liquid of the same conductivity.
- Repeat trial as indicated in Table 1 and in the subsequent trials, increaring the mixing time and amount of tracer as indicated in the tables below.

Trial 1&2	**25 wt% NaCl (mL)**			
			Trial 1	**Trial 2**
		Viscosity (cP)	1	33
		Mixing Speed (rpm)	50	80
		Sampling time during mixing (min)		
Run				
1	94		0.5	0.5
2	188		3	3
3	376		5	5
4	752		10	10
5	1,504		15	30

Table 1: Mixing duration and volume of tracer added for Trials 1 and 2
Trial 1 = Viscosity 1 cP agitation, 50 rpm and
Trial 2 = Viscosity 33 cP-agitation, 80 rpm

Trial 3	**25 wt% NaCl (mL)**		
			Trial 3
		Viscosity (cP)	33
		Mixing Speed (rpm)	100
		Sampling time during mixing (min)	
Run			
1	188		5
2	376		10
3	752		15
4	1504		20
5	3,008		20
6	6,016		20

Table 2: Mixing duration and volume of tracer added for Trial 3
(Viscosity 33 cP, agitation 100 rpm)

Results

Homogeneity results are dependent on solution viscosity, agitation speed, and the duration of the mixing.

For the 1 cP solution mixed at 50 rpm, a good homogeneity between the cup and the bulk conductivity is achieved in 0.5 minutes, as shown by a concentration ratio of 105 percent.

For the 33 cP solution mixed at 80 rpm, homogeneity is not achieved after 30 minutes of mixing with only an 89 percent concentration ratio.

Increasing the agitation speed to 100 rpm enables the 33 cP solution to reach an excellent homogeneity in 20 minutes with a ratio of 91 percent, 95 percent, and 99 percent.

Results are presented in the tables below.

Trial 1	Mixing Time After NaCl Charge (min)	25 wt.% NaCl (mL)	Bulk Conductivity (µS/cm)	Cup Conductivity (µS/cm)	% of Bulk Increase
Run					
1	0.5	94	1,079	1,137	105
2	3	188	2,079	2,017	97
3	5	376	4,070	4,040	99
4	10	752	7,170	7,160	100
5	15	1,504	12,180	12,220	100

Table 3: Conductivity of the liquid bulk and conductivity in the cup after addition of solute tracer and incremental mixing steps at 50 rpm in a 1 cP solution.

33 cP viscosity, 80 rpm (PEG solution) test

Trial 2	Mixing Time After NaCl Charge (min)	25 wt.% NaCl (mL)	Bulk Conductivity (µS /cm)	Cup Conductivity (µS /cm)	% of Bulk Increase
Run					
1	0.5	94	1,643	1,958	27
2	3	188	2,110	2,810	18
3	5	376	3,270	4,400	29
4	10	752	5,910	7,410	50
5	20	752	9,700	10,500	74
6	30	1,504	15,500	16,100	89

Table 4: Conductivity of the liquid bulk and conductivity in the cup after addition of solute tracer and incremental mixing steps at 80 rpm in a 33 cP solution.

33 cP viscosity, 100 rpm (PEG solution) test

Trial 2	Mixing Time After NaCl Charge (min)	25 wt.% NaCl (mL)	Bulk Conductivity (µS /cm)	Cup Conductivity (µS /cm)	% of Bulk Increase
Run					
1	5	188	2,360	3,000	33
2	10	376	3,660	4,890	35
3	15	752	6,680	8,400	51
4	20	1,504	14,650	14,700	99
5	20	3,008	25,500	26,600	91
6	20	6,016	46,900	47,900	95

Table 5: Conductivity of the liquid bulk and conductivity in the cup after addition of solute tracer and incremental mixing steps at 100 rpm in a 33 cP solution.

Percent of Bulk Conductivity Achieved in Cup Sample

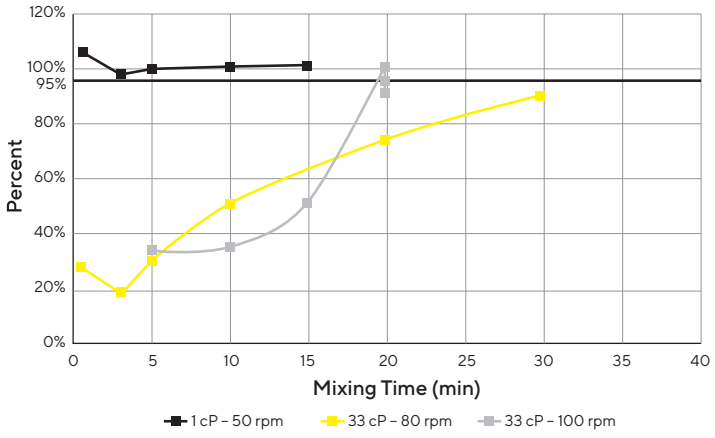

Figure 6: Homogeneity of the liquid in the cup compared to the bulk of the mixing bag for 1 cP and 33 cP solutions as a function of the mixing duration. Mixing speed: 50rpm (1cP), 80 rpm (33 cP) and 100 rpm (33 cP)

Discussion

Results captured in Figure 6 show that in the case of the 1 cP solution (Trial 1), the cup conductivity was within 97 percent of the bulk conductivity after three minutes of mixing. In the 33 cP solution at 80 rpm (Trial 2), the conductivity in the cup was within 89 percent of bulk solution conductivity after 30 minutes.

In Trial 3, performed with a 33 cP solution but with higher agitation speed of 100 rpm, the cup conductivity was able to reach 95 percent of bulk conductivity after 20 minutes of mixing. "Well-mixed" refers to conductivity measurements within 95 percent of one another. Higher mixer speed contributed to faster equilibration between bulk and cup conductivity.

The 33 cP solution represents a worst-case scenario of viscosity for the low-pH viral inactivation step. In working conditions with actual viral inactivation, low-viscosity solutions achieve complete homogenization after 30 seconds.
With low-pH viral inactivation, the pH of the downstream intermediate is first reduced and maintained at a low pH of approximately 3.6 – 3.7 for a validated period or until the virus is totally inactivated. The additional time required for complete homogenization in the cup is shorter than that required for complete viral inactivation — usually one hour.

Conclusion

This study demonstrates the suitability of the Flexsafe® Pro Mixer for viral inactivation with similar solute concentration in the cup and in the bulk of the single-use bag in both low-viscosity and high-viscosity solutions. Any stagnant zone in the cup had minimal impact, showing the Flexsafe® Pro Mixer is suitable for the viral inactivation process step.

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