

Liquid-Liquid Mixing Without Vortex Formation

In the 50 L Allegro™ Mixer, the 100 L and 400 L LevMixer® System, and the 1500 L Magnetic Mixer



Example of various mixing tank size scales for the LevMixer system and Magnetic Mixer: 650 L, 400 L, 200 L and 2000 L.

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1 Introduction

Mixing of miscible liquids of similar characteristics is a common requirement in bioprocessing. Pall and a customer joined forces to determine appropriate mixing conditions for selected mixing technologies. For such processes, the typical requirement is that the mixing impeller speed be high enough to reach homogeneity in a generally acceptable timeframe (≤ 5 minutes), while being low enough to avoid vortex formation (which can damage products sensitive to air-liquid interface interactions).

This application note describes liquid-liquid homogenization conditions which meet these requirements at various filling levels in the 50 L Allegro mixer, the 100 L LevMixer system, the 400 L LevMixer system and the 1500 L Magnetic Mixer.

Magnetic Mixer and LevMixer systems utilize the same tanks, with mixing impellers with the same dimensions. Therefore, the results from the LevMixer system are also deemed applicable to the Magnetic Mixer system and vice versa.

In order to cover most typical biotech sensitive applications, the tests were conducted under stringent conditions in terms of density of the spiking solution and bulk. As the impellers are bottom mounted, addition from the top of a low-density liquid to a high-density bulk solution was considered to be a worst-case scenario. Glucose (24.3% w/w aqueous solution, density of 1.098 kg/L) was chosen as the bulk solution, and NH_4Cl (10% w/w aqueous solution, density of 1.029 kg/L) was used as the spiking solution.

2 Materials and Methods

2.1 Materials

Table 1

Materials

Description	Part Number
Allegro mixer 50 L system	LGR-EP8686-50
Magnetic Mixer drive unit	DU006-EU
LevMixer drive unit	LT-DBTL300
Magnetic Mixer / LevMixer Tanks	
100 L	LM100JCMA001
400 L	LM400JCMA001
1500 L	LM1500JCMA003
Biocontainers	
50 L	7403-1056M (corresponding to standard system part number: 609-100R)
100 L	7403-1056V (corresponding to standard system part number: 7403-1626T)
400 L	7403-1056U (corresponding to standard system part number: 7403-1626Y)
1500 L	9404-1028V (corresponding to standard system part number: 7404-1483S)
Chemicals	
Ammonium chloride	Merck* reference number 101142.5000
Glucose anhydride	Merck reference number 137048.9012
Polysorbate 80	Avantor* reference number 4117-06

Table 1 continued

Accessories

Conductivity probes	120 mm conductivity sensor, ISM, Mettler-Toledo*, InPro7100/12/120/4435
Transmitter	Mettler M300 transmitter 2 channels
Timer	Kalenji* ONstart 310

2.2 Methods

For each mixing tank, the biocontainer was installed, inflated and then cut open at the top for better visibility of the mixing process. As presented in Figure 1, two conductivity sensing probes were inserted into the biocontainers:

- At the bottom, through the front probe fitment
- At the top, in the front corner (at a depth of 5-10 cm below the liquid surface)

The bulk solution, containing 24.3% glucose w/w and 0.05% Polysorbate 80 w/w, was prepared in advance. After starting the timer and data logging on the transmitter, the biocontainer was filled to various volumes with the bulk solution and stirred at various speeds. Each mixing test started at nominal maximum volume (e.g. 400 L in a 400 L biocontainer), and then the volume was reduced stepwise for successive tests. In each test, the vortex was evaluated by viewing it from the top and through the door window. After mixing for at least 1 minute (to reach a steady-state), spike additions of 0.1% of tank fill volume were made with NH₄Cl 10% solution, with the addition being made to the top corner opposite the top conductivity probe (see Figure 1) from a height of 5 to 20 cm above the liquid surface. The mixing was continued for at least 5 minutes before the next mixing conditions were set. If homogenization time or vortex formation were not adequate, the mixing speed was adapted accordingly.

Based on Dechema recommendations (Meusel *et al*, 2016¹), 95% and 99% homogenization times were defined as the time elapsed after the addition so that all sensor readings plateaued within $\pm 5\%$ and $\pm 1\%$ respectively of their signal shifts. An example of homogenization time determination is presented in Figure 2. Air-liquid interfaces were evaluated by visually confirming the absence of a significant vortex formation (< 3 cm depth) and the absence of foaming.

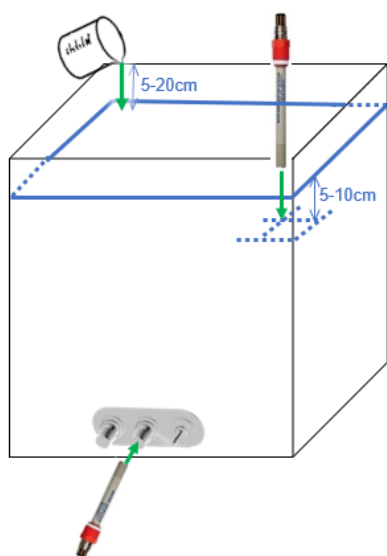


Figure 1

Location of liquid addition, probe insertion in the biocontainer and operator visibility.

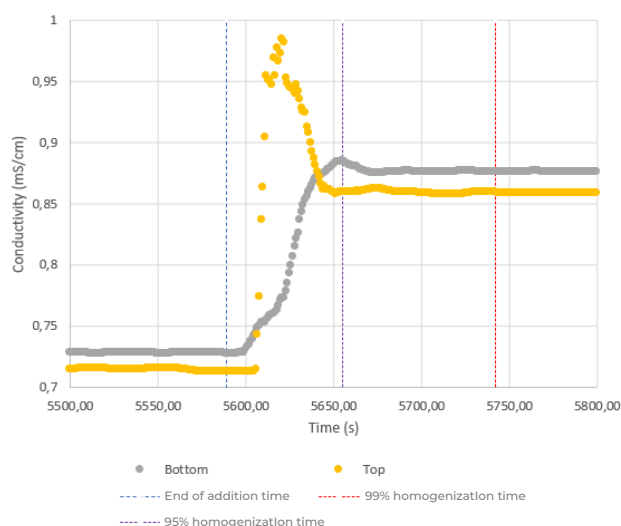


Figure 2

Example of homogenization time determination - 1500 L Magnetic Mixer 100% 180 rpm 3rd replicate: 99% homogenization time is 2.55 minutes and 95% homogenization time is 1.10 minutes.

3 Results

The homogenization times are reported in Table 2. Tests were conducted under conditions which avoided significant vortex formation.

Table 2

Homogenization times based on conductivity in different technologies and tank sizes at various mixing speeds and filling levels.

Tank Size (L)	Mixer Technology	Filling Level	Mixing Speed (rpm)	99% Homogenization Time (mins)	95% Homogenization Time (mins)
50	Allegro Mixer	100%	46	0.65	0.53
50	Allegro Mixer	100%	46	0.70	0.53
50	Allegro Mixer	100%	46	0.72	0.52
50	Allegro Mixer	100%	39	1.53	0.67
50	Allegro Mixer	44%	55	0.48	0.32
50	Allegro Mixer	44%	39	0.72	0.53
50	Allegro Mixer	18%	39	1.20	0.43
50	Allegro Mixer	8%	39	0.92*	0.28*
50	Allegro Mixer	4%	39	0.97 ^v	0.88 ^v
100	LevMixer System	100%	50	0.77	0.60
100	LevMixer System	100%	50	0.72	0.65
100	LevMixer System	100%	42	0.85	0.63
100	LevMixer System	44%	42	0.87	0.57
100	LevMixer System	44%	42	0.73	0.52
100	LevMixer System	44%	42	0.75	0.43
400	LevMixer System	100%	101	1.20	0.90
400	LevMixer System	100%	73	2.03	1.02
400	LevMixer System	100%	73	1.97	1.50
400	LevMixer System	100%	73	1.88	1.43
400	LevMixer System	60%	73	1.55	0.78
400	LevMixer System	60%	65	1.20	0.77
400	LevMixer System	60%	65	1.43	1.03
400	LevMixer System	35%	65	1.17 [†]	0.68 [†]
400	LevMixer System	35%	58	1.02	0.78
400	LevMixer System	35%	58	1.23	0.78
400	LevMixer System	35%	58	1.23	0.78
400	LevMixer System	15%	58	1.48	0.98
400	LevMixer System	15%	58	1.23	0.73
400	LevMixer System	15%	58	0.92	0.82

Table 2 continued

Tank Size (L)	Mixer Technology	Filling Level	Mixing Speed (rpm)	99% Homogenization Time (mins)	95% Homogenization Time (mins)
1500	Magnetic Mixer	100%	180	3.75	2.55
1500	Magnetic Mixer	100%	180	2.55	1.10
1500	Magnetic Mixer	97%	101	6.42	5.12
1500	Magnetic Mixer	55%	100	1.87	1.23
1500	Magnetic Mixer	55%	100	1.68	1.33
1500	Magnetic Mixer	52%	161	1.10	0.88
1500	Magnetic Mixer	52%	161	1.00	0.73
1500	Magnetic Mixer	52%	161	0.97	0.77
1500	Magnetic Mixer	40%	82	2.17	1.10
1500	Magnetic Mixer	40%	82	1.65	0.92
1500	Magnetic Mixer	40%	50	4.42	3.87
1500	Magnetic Mixer	40%	50	3.78	2.78
1500	Magnetic Mixer	27%	43	3.53	2.90
1500	Magnetic Mixer	27%	43	3.08	2.18
1500	Magnetic Mixer	27%	43	3.38	1.65
1500	Magnetic Mixer	8%	43	4.58	2.98
1500	Magnetic Mixer	8%	43	3.57	2.23

* Only the bottom probe was used.

‡ Only the top probe was used and even initially no stable conductivity signal could be collected so that empiric homogenization times were chosen.

† Intermittent vortex formation >3 cm <50% of the time was observed.

4 Conclusion

Mixing of miscible liquids with differing densities was characterized using conductivity sensors and visual observation in four Pall single-use mixing systems: Allegro mixer/50 L cubical tank, LevMixer system/100 L cubical tank, LevMixer system/400 L cubical tank and Magnetic Mixer/1500 L cubical tank. Under the conditions tested, homogenization times ranged from 0.32 minutes to 4.58 minutes without vortex formation, thus satisfying the target requirement of ≤ 5 minutes.

Further information on the test parameter selection, discussion of the results and conclusions (e.g. applicable mixing speed and time) is presented in report SLS-SUT-20210603-ADB-01 which can be obtained from your Pall contact.

5 References

1. W. Meusel *et al*, Recommendations for Process Engineering Characterisation of Single-Use Bioreactors and Mixing Systems by Using Experimental Methods, *Dechema*, January 2016.
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Corporate Headquarters

Port Washington, NY, USA
+1-800-717-7255 toll free (USA)
+1-516-484-5400 phone

European Headquarters

Fribourg, Switzerland
+41 (0)26 350 53 00 phone

Asia-Pacific Headquarters

Singapore
+65 6389 6500 phone

Visit us on the Web at www.pall.com/biotech

Contact us at www.pall.com/contact

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USD3560 – Nov 2021