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White Paper

Performance Characteristics of the Mobius® ADC Reactor for Conjugation

Antibody-drug conjugates (ADCs) deliver a unique combination of specificity and potency. The monoclonal antibody (mAb) targets a surface antigen on cancer cells, delivering a highly cytotoxic small molecule payload to the tumor with high precision. The pace of ADC development and approvals is accelerating, and this modality has become an increasingly important class of drugs in many biopharmaceutical pipelines.

In an ADC construct, the mAb and payload are connected via a linker which modulates ADC stability in the circulation as well as release of the payload. A critical step in the production of ADCs is the conjugation in which the linker, attached to the payload (typically a small highly active pharmaceutical ingredient), is tethered to the mAb. Because the payload is a highly potent, toxic agent, the conjugation step requires special consideration for safe handling and containment. Historically, this step has been performed in glass or stainless-steel reaction vessels or mixers.

However, adoption of single-use technologies is growing due to the realized benefits such as decreased risk of contamination, ease of scalability, reproducibility, flexibility, and reduced costs and time savings due to process efficiencies related to setup, cleaning validation, and actual cleaning.

This white paper describes a series of studies using a model fluid to evaluate the mixing performance of Mobius® ADC Reactor for conjugation including heating and cooling times, acid/base blend times, and effects of mixing speeds for 10 L, 100 L, and 500 L sizes (Figure 1).

Results of these studies provide a starting point for comparison to existing glass and stainless-steel reactors and can serve as guidelines to assist users in the integration of this single-use technology into the ADC manufacturing workflow.

Figure 1.
10 L, 100 L, and 500 L single-use Mobius® ADC Reactors (left to right).

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Mixing Performance

Heating and Cooling Times

The ability to control the temperature is essential for a successful conjugation. A study was therefore conducted to determine the amount of time required for heating and cooling different volumes of USP water at various mixing speeds (revolutions per minute; RPM) in the Mobius® ADC Reactors. Table 1 summarizes results for the 10 L, 100 L, and 500 L reactors with starting temperatures of 24.2–25.0 °C for the heating study and 36.5–37.7 °C for the cooling study.

For all reactor sizes, increasing RPMs reduced heating times; in contrast, higher RPMs had little to no effect on cooling times. Regardless of the mixing speed, larger volumes required less heating time but more time to cool.

10 L

Volume [L]	TCU [°C]	Start Temp [°C]	End Temp [°C]	Speed [rpm] & Time [min]			
				150	400	600	700
2.5	38.5	24.2	36.6	159	114	N/A	N/A
4	38.5	24.2	36.6	140	80	66	N/A
10	38.5	24.2	36.6	107	76	55	51

Volume [L]	TCU [°C]	Start Temp [°C]	End Temp [°C]	Speed [rpm] & Time [min]			
				150	400	600	700
2.5	25.0	36.5	25.3	126	134	N/A	N/A
4	25.0	37.7	25.3	164	136	186	N/A
10	25.0	37.7	25.3	114	136	106	104

100 L

Volume [L]	TCU [°C]	Start Temp [°C]	End Temp [°C]	Speed [rpm] & Time [min]			
				100	200	300	400
20	38.5	24.9	37.0	250	162	138	120
50	38.5	25.0	37.0	174	146	136	126
100	38.5	25.0	37.0	140	120	114	106

Volume [L]	TCU [°C]	Start Temp [°C]	End Temp [°C]	Speed [rpm] & Time [min]			
				100	200	300	400
20	25.0	37.0	25.3	184	174	174	232
50	25.0	37.0	25.3	224	210	212	236
100	25.0	37.0	25.3	238	216	204	218

500 L

Volume [L]	TCU [°C]	Start Temp [°C]	End Temp [°C]	Speed [rpm] & Time [min]			
				100	150	200	250
100	38.5	25.0	37.0	80	71	66	59
250	38.5	25.0	37.0	88	83	80	75
500	38.5	25.0	37.0	111	110	104	101

Volume [L]	TCU [°C]	Start Temp [°C]	End Temp [°C]	Speed [rpm] & Time [min]			
				100	150	200	250
100	25.0	37.0	25.3	94	91	96	145
250	25.0	37.0	25.3	138	136	140	155
500	25.0	37.0	25.3	197	190	191	201

Table 1.

Summary of heating and cooling times respectively left and right for 10 L, 100 L, and 500 L Mobius® ADC Reactors. Data were not recorded (N/A) if splashing, bubbling, dimpling, or vortexing were observed.

Figure 2.

500 L Mobius® ADC Reactor with operators installing the single-use assembly.

Blending Times

In addition to heating and cooling, uniform mixing is essential during ADC conjugation. Similar blending time should also be kept when the process is scaled up. To assess the mixing performance of the Mobius® ADC Reactors, a study was conducted in which acid and base were added into the carriers and the time required to reach a stable pH measured. Stable pH was defined as the point at which the measured valued was consistent at to three decimal points for ≥3 seconds. Table 2 summarizes the study results and shows that as mixing speeds increased, the average blend time decreased across all reactor sizes; at higher RPMs however, mixing speed had less of an effect. As volume increased, the average blending time also increased.

10 L

Volume [L]	Capacity [%]	Mixing Speed [rpm]						
		150	200	300	400	500	700	900
2.5	25	28	25.5	20	19	X	X	X
4	40	32	25	22	20	19	X	X
6	60	36	30	23	21	20	19	X
8	80	40	31	27	23	24	18	X
10	100	50	41	30	24	23	X	X

Note: Maximum mixing speed is 1000 rpm.

100 L

Volume [L]	Capacity [%]	Mixing Speed [rpm]				
		150	200	300	400	500
20	20	36	23	20	19	X
40	40	53	26	24	21	20
60	60	62	35	26	22	19
80	80	99	46	34	25	21
100	100	113	50	33	25	20

Note: Maximum mixing speed is 500 rpm.

500 L

Volume [L]	Capacity [%]	Mixing Speed [rpm]				
		50	100	150	200	250
75	15	49	29	27	25	X
125	25	54	31	27	26	22
175	35	62	41	30	25	22
225	45	X	45	35	27	24
300	60	X	53	41	33	29
375	75	X	65	50	37	30
450	90	X	82	60	44	32
500	100	X	84	60	44	35

Note: Maximum mixing speed is 250 rpm.

- Good
- Slight dimpling, may include bubbling
- Slight vortexing or dimpling/splashing/bubbling
- Vortexing/severe splashing/bubbling

Table 2.

Summary of blend times and mixing effects for 10 L, 100 L, and 500 L Mobius® ADC Reactors.

Effect of Mixing Speed

Acid and base blend times of the 10 L, 100 L, and 500 L Mobius® ADC Reactors are shown in graphical form in Figure 2 for 100% volume (A), 60% volume (B) and 25% volume (C), respectively. Blend times at the same relative volume (% of nominal volume) at varying mixer motor output (% of maximum output) are compared to show similar mixing profiles across the three reactor sizes when operating at the same normalized conditions.

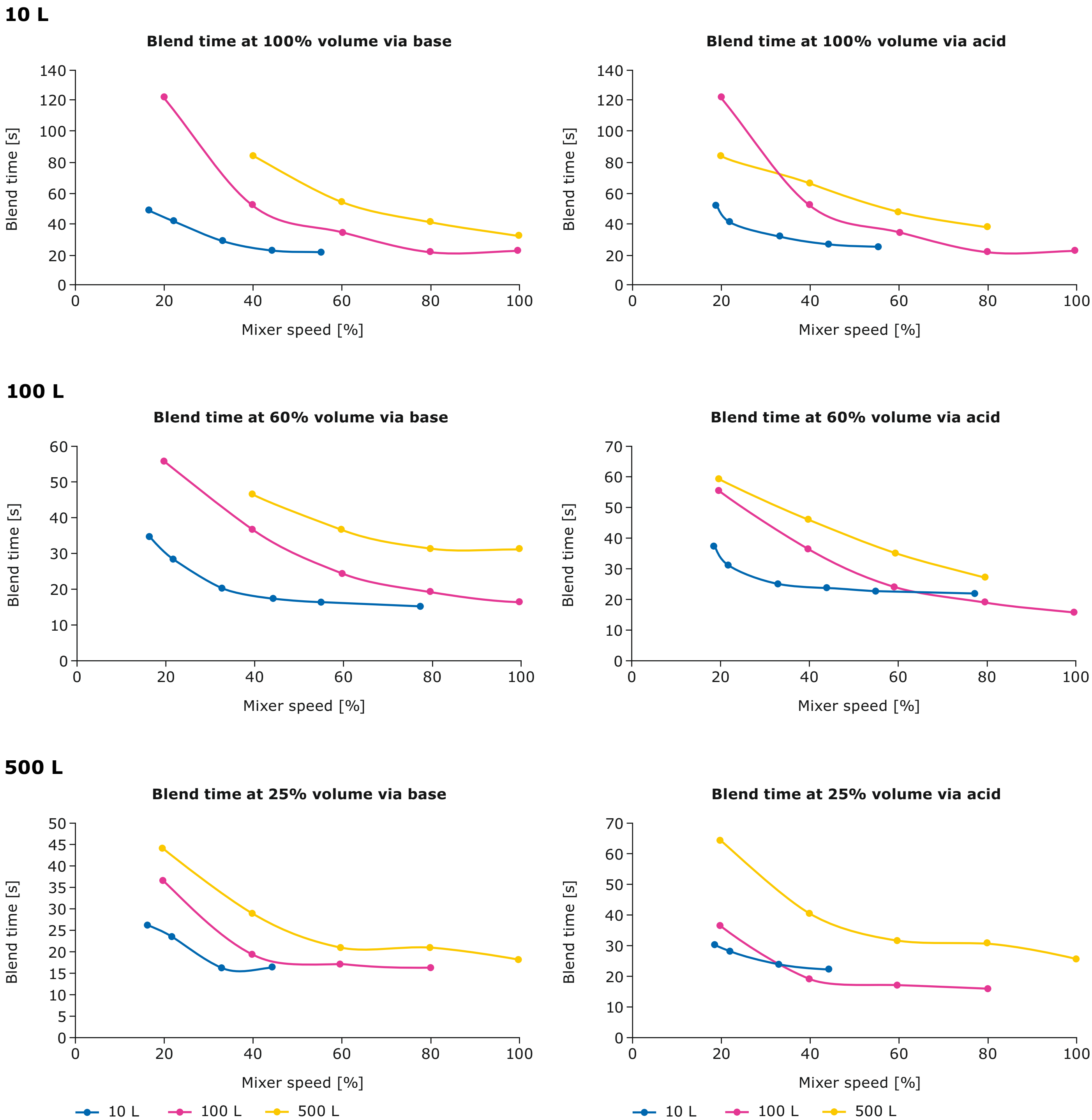
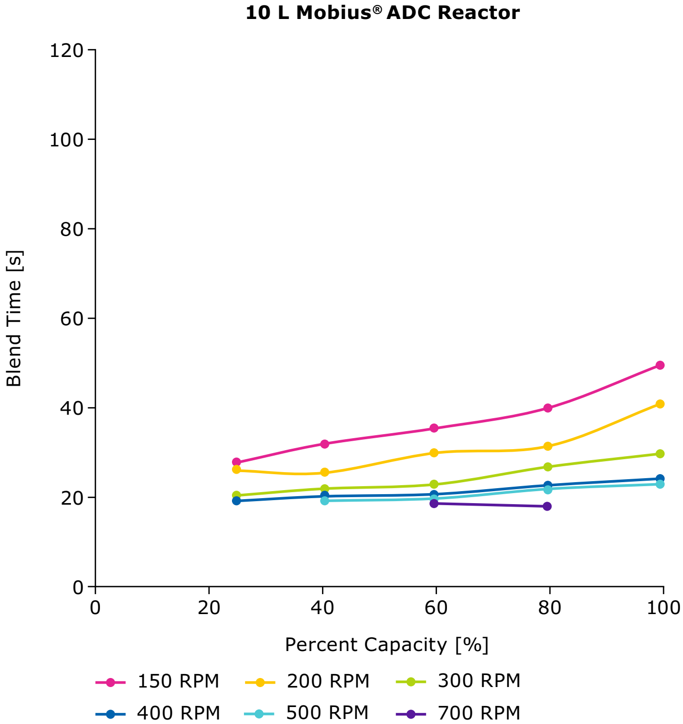


Figure 2.

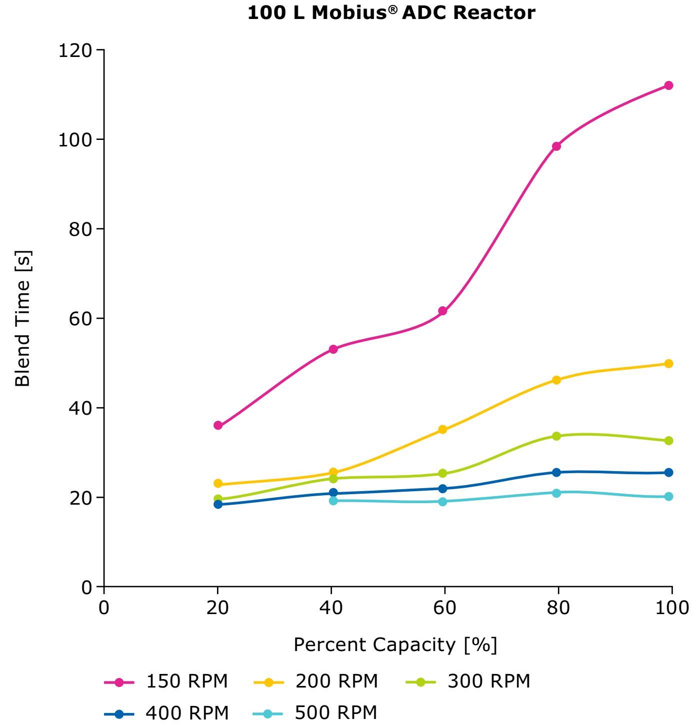
Average acid/base blend time as a function of mixer speed (%) 10 L, 100 L, and 500 L Mobius® ADC Reactors at various volumes.

Blend times of the 10 L, 100 L, and 500 L Mobius® ADC Reactors are shown in graphical form in Figure 3. Blend times at varying mixing speeds are compared to show mixing profiles when operating at the same percent capacities (% of nominal volume).

10 L



100 L



500 L

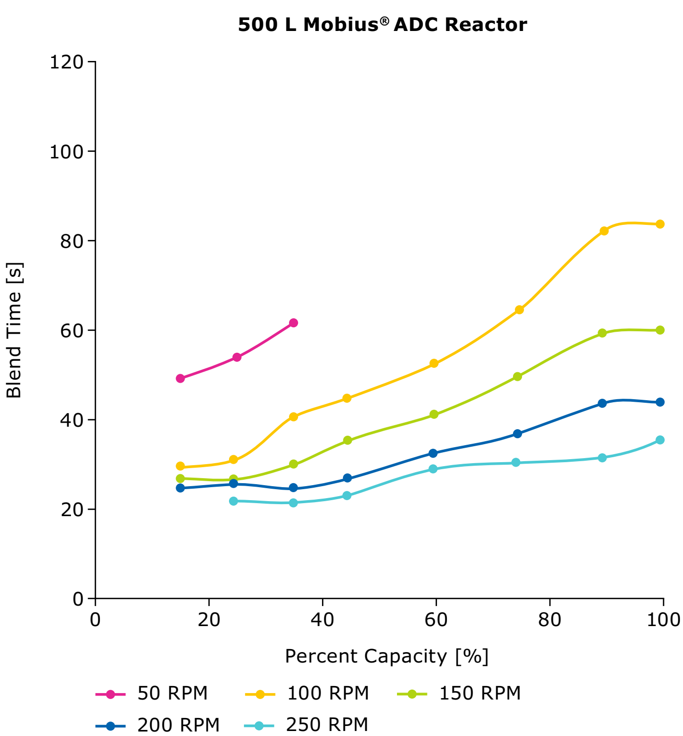


Figure 3.

Average acid/base blend time as a function of percent capacity for 10 L (A), 100 L (B), and 500 L (C) Mobius® ADC Reactors at various speeds.

Conclusion

ADCs are being increasingly used as first-line cancer therapies and in combination with other drugs. The precision targeting enabled by the antibody portion, together with the potency of the cytotoxic payload, create a modality with a broad scope of possible therapeutic indications.

Single-use technologies such as Mobius® ADC Reactors for conjugation offer many advantages for the unique challenges presented by the ADC manufacturing workflow. The risk of contamination and the risk to operators can be significantly reduced while scalability, reproducibility, and flexibility can all be increased.

The results of the studies described above demonstrate the performance of the Mobius® 10 L, 100 L, and 500 L ADC Reactors in terms of heating and cooling times, acid/base blend times, and effects of mixing speeds. These data offer a starting point for comparison to existing glass and stainless-steel reactors and the development of new processes that incorporate single-use technologies for ADC conjugation.

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