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Tech Brief

Mobius® ADC Reactor Performance

With their ability to target specific cells and minimize off-target toxicity, antibody drug conjugates (ADCs) are being leveraged to treat serious diseases including cancer, with breast cancer and non-small-cell lung cancer leading the way. These complex biologics consist of a targeting antibody, a cytotoxic payload, and a linker. The linker attaches the payload to the antibody and is designed to be stable in circulation and often releases the payload once the ADC is inside the targeted cells.

The global market for ADCs is experiencing rapid growth, with a substantial number of ADC drugs in the clinical pipeline and several ADCs already approved by the FDA. The increasing prevalence of cancer patients and the expanded therapeutic window offered by ADCs are among the key drivers of this growth. With a deluge of new ADC projects underway, simplifying development and manufacturing processes will be advantageous for the pharmaceutical industry, enabling the delivery of new therapies to patients at an accelerated pace.

Background

The ADC conjugation step, during which the ADC construct is synthesized by linking mAb, linker and payload, is typically performed using either glass or stainless-steel reactors depending on the scale and phase of development. Elsewhere in the process, single-use components – filters, disposable flow paths, etc. – have been used successfully for years.

The numerous benefits of using single-use systems under GMP include among others operator safety, decreased risk of cross-contamination, flexibility, time saving through efficiencies such as easy setup and cleanup with no need for cleaning validation.

Despite the numerous advantages of using single-use systems, there are several challenges and risks associated with their application in ADCs processing. We have meticulously addressed these risks through careful design and thorough testing.

A dedicated ADC reactor

The Mobius® ADC Reactor is designed to meet the precise demands of bioconjugation. The single-use assembly is made of damage-resistant Ultimus® film. Containing a woven nylon layer, Ultimus® film acts as a protective barrier against abrasion, impacts, tears, and material fatigue, therefore providing additional leak prevention while maintaining the ease of handling. The ADC Reactor single-use assembly leverages the Mobius® MIX technology with Levitronix® agitation, providing gentle mixing tailored to the bioconjugation process. The single-use assembly includes specific ports for solvent and monoclonal antibody (mAb) addition, designed to minimize splashing and foaming, thereby preserving product quality during the mAb addition process (Figure 1).

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The challenges associated with employing single-use components in ADC processing are primarily related to chemical compatibility due to the use of potent solvents in ADC production. Extensive testing has been conducted, including gamma irradiation of all components before chemical compatibility testing, to replicate real-use conditions. The molded plastic components, tubing, gaskets, and Ultimus® film used in the Mobius® ADC Reactor are all compatible with concentrations of DMSO and DMAc that exceed typical conjugation conditions.[1]

To accommodate the specific assembly, the ADC reactor carrier includes a swinging rail for supporting solvent tubes and vent filter and a side opening or the mAb addition tubing (Figure 2).

The Mobius® ADC Reactors are available in three sizes: 10 L, 100 L and 500 L covering working volumes from 4 to 500 L.

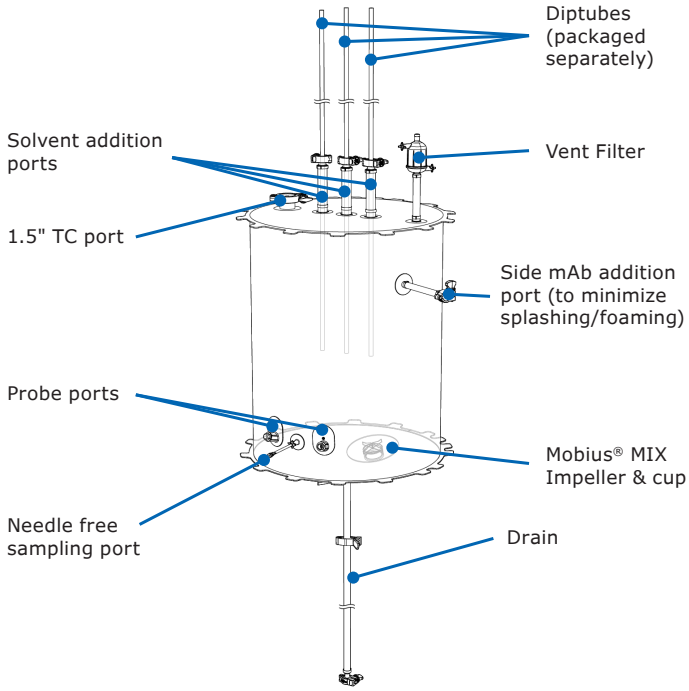
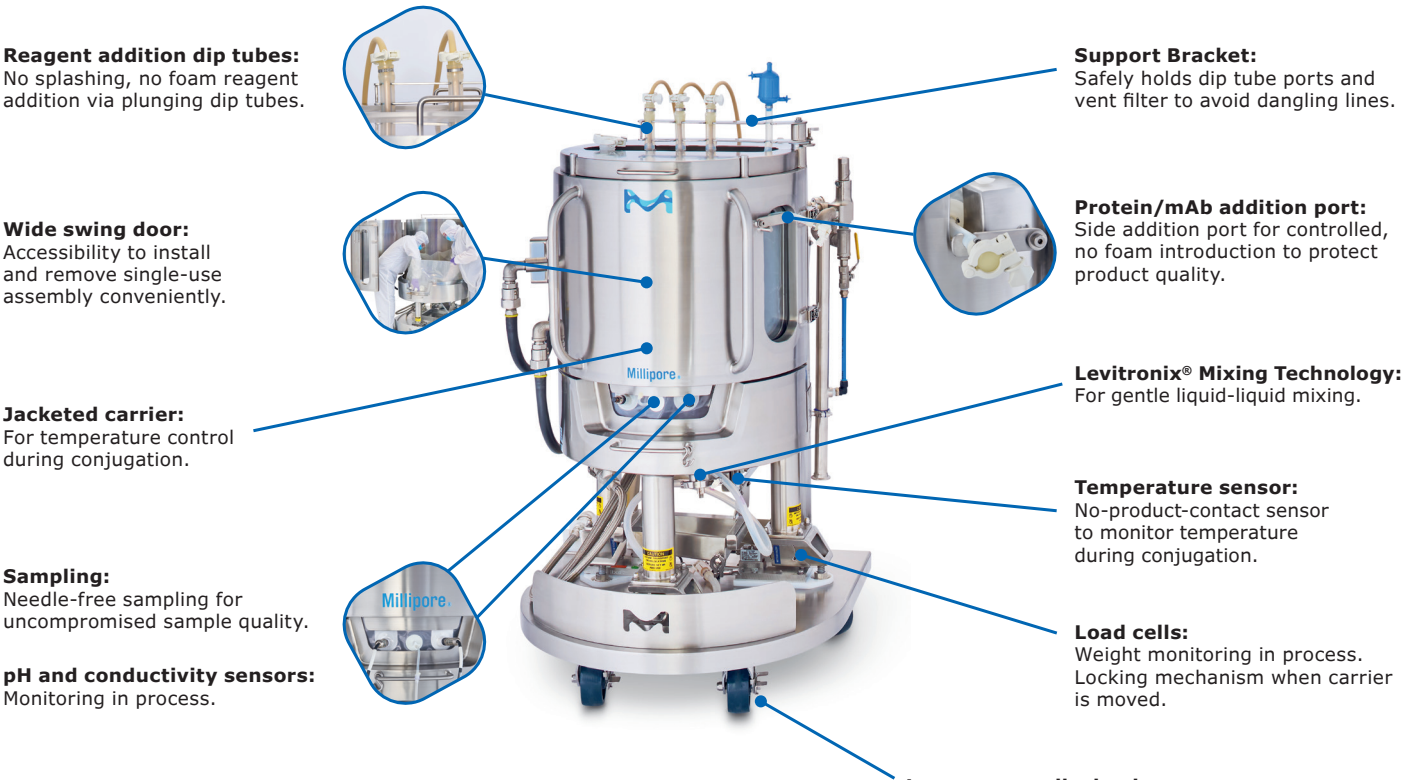


Figure 1.

Design of the 100 L and 500 L assemblies.



Note: The temperature sensor differs for the 10 L scale. Refer to the data sheet for more details.[2]

Figure 2.

Mobius® ADC Reactor 100 L.

Mixing performance

The mixing performance of the Mobius® ADC Reactor is comparable to glass or stainless-steel vessels, which are typically used for conjugation. In a study we conducted and summarized in a separate document, a model solution was used to characterize mixing efficiency and temperature control (heating and cooling) at different volumes and mixing speeds to assist users in the integration of using this single-use technology in their manufacturing workflow. In the current application note, ADC manufacturers shared their feedback on real conjugation applications.

User testing

To evaluate the design and the efficiency of the Mobius® ADC Reactor in real conditions, two customers evaluated the 10 L and the 500 L systems respectively at their own facilities and following their proprietary processes. The subsequent sections showcase their respective findings.

Customer A

Customer A submitted the 10 L system to different tests. The characteristics of the 10 L reactor are summarized in Table 1.

Working volumes	1–10 L
Minimum volume for sampling and sensors	3.5 L
Mixing speed range	0–1000 rpm
Operating Temperature Range	4 to 60 °C

Table 1.

10 L reactor specifications.[3]

Reactor pressurization

Protein conjugation frequently requires the protein to be in a specific oxidation state (e.g. reduced disulfide bridges to accommodate reaction with succinimide functions). To preserve the oxidation state of the protein, reactions may be conducted under nitrogen atmosphere. During the present testing, the bag was pressurized, via the vent filter included in the assembly, with 15–20 mbar of nitrogen using a pressurization/venting setup as illustrated in Figure 3 which comprised a pressure regulator set to supply nitrogen at 15 mbar and a backpressure regulator configured to vent gas at 20 mbar. This setup ensured the bag was moderately inflated, maintaining good contact with the carrier walls and enhancing the fit of the bag within the carrier. During liquid removal, the bag maintains its shape, while any excess gas is expelled through the second valve during liquid addition.

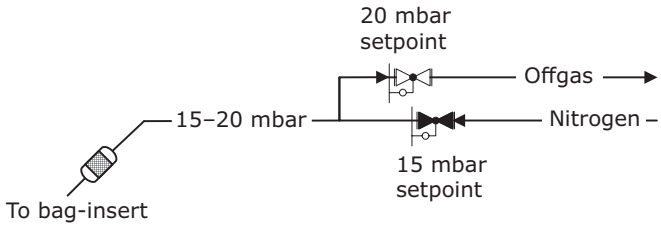


Figure 3.

Pressurization setup.

Heat transfer

Several reactions involved in ADC synthesis can be exothermic – addition of DMSO for instance – therefore to ensure the operators' safety and maintain the quality of the ADC product, it is critical to control the temperature of the reactor. The heat transfer depends on the ADC reactor but as well on the Thermal Control Unit used for the test. Heat transfer was assessed using step changes of the heating fluid supplied to the jacket of the bag holder: 20 to 30 °C, and 30 to 40 °C.

The following diagram (Figure 4) shows heating curves at a filling level of 10 kg (water), using three different agitation speeds (200, 500 and 800 rpm). Heat exchange at 200 rpm was slower compared to heat exchange at 500 and 800 rpm while it was very comparable between 500 and 800 rpm. Based on these data, a heating power of 2.5 to 3.0 W/kg.K can be calculated at 500–800 rpm and a filling level of 5–10 kg.

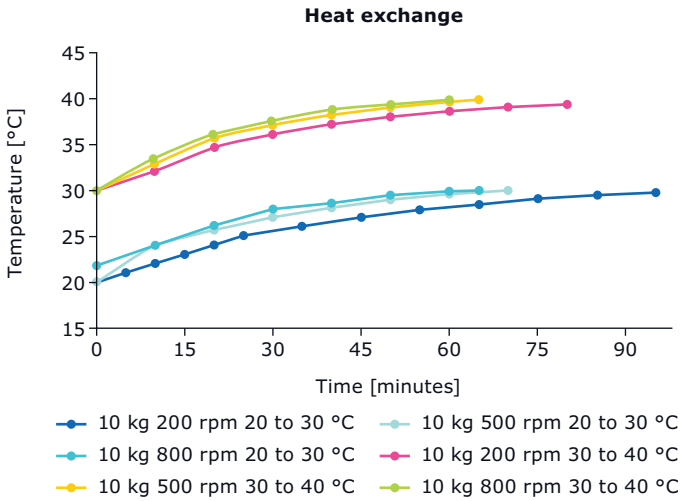


Figure 4.

Temperature (°C) versus heating time (minutes) at 3 different agitation rates.

Mixing performance

Mixing performance was assessed in a semi-quantitative manner by addition of 10% NaOH solution into a solution of succinic acid containing phenolphthalein as a pH indicator, allowing for visual assessment of the mixing by the disappearance of the purple color of phenolphthalein at a pH ≥10.

Mixing test 1:
Addition of a bolus to measure dispersion times

To demonstrate the dispersion time post bolus addition into the vessel, a 10 L solution of succinic acid with a small quantity of phenolphthalein (added as an ethanolic solution) was prepared. Following this, 60 mL of a 10% Sodium hydroxide solution was added in a single bolus (within 3 seconds), and the time until the purple color disappeared was recorded. The graph below (Figure 5) illustrates the dependency of dispersion time on the agitation rate at a 10 L working volume, when the base is added on the surface above the agitator. It is worth noting that values for surface addition distant from the agitator (data not shown) exhibited insignificant variations, underscoring the efficiency of the mixing no matter the choice of the bolus addition port.

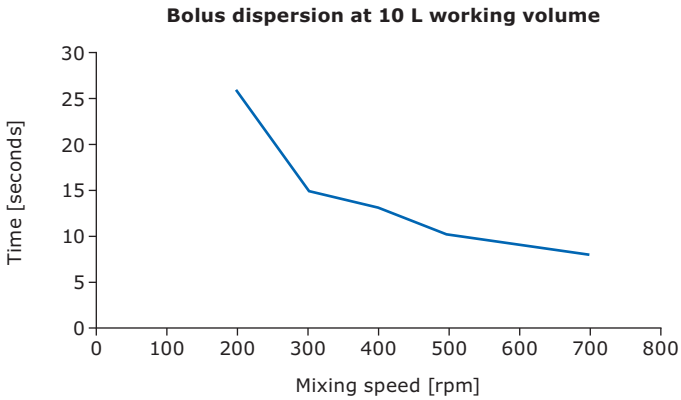


Figure 5.

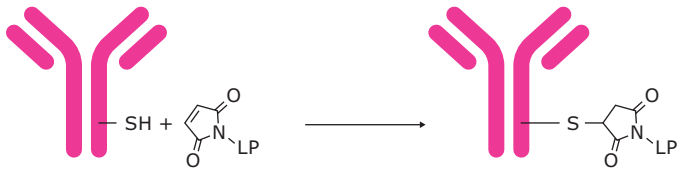
Mixing time at different agitation rates.

Mixing test 2:
Cavitation/Foam formation

Biological molecules are prone to denaturation at liquid/gas interfaces, and the formation of foam can easily occur in liquids containing such biological material if gas becomes trapped within the liquid. Consequently, in an effort to test the reactor's limits, 2.5 kg of a detergent solution (Cosa CIP 90 cleaner, 2% solution) were introduced into the reactor and agitated. The solution started foaming at an agitation rate of approximately 400–500 rpm. However, this filling volume falls below the recommended minimum working volume (4 L), thus only highlighting the necessity for careful consideration of the agitation speed during the draining phase, should the mixer need to remain operational.

Execution of an example coupling reaction

A coupling reaction (thiol addition to a succinimide residue) was conducted between a monoclonal antibody and a linker-payload type molecule (LP), see Figure 6 below. The choice of the test system was based on the availability of testing materials rather than any known specific critical process or equipment issues.



LP = Linker-Payload

Figure 6.

Schematic overview of reaction conducted.

Setup

The setup used is illustrated in the following overview diagram (Figure 7). After installation of the bag in the carrier, the bag was connected to the 15–20 mbar nitrogen/venting system previously described to allow for proper adherence of the bag surfaces to the bag holder walls to optimize the heat transfer.

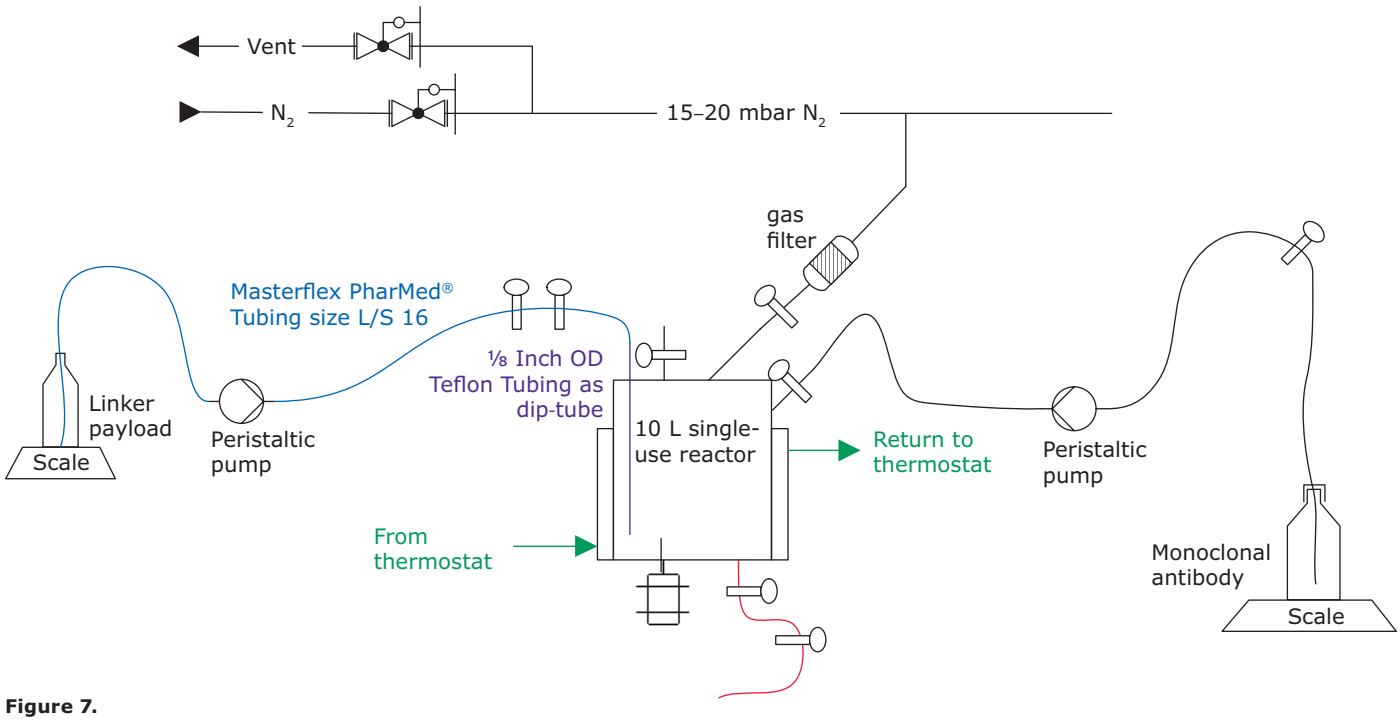


Figure 7.

Overview of the setup.

Execution of Reaction

A solution of 200 grams of monoclonal antibody (approximately 4 liters) was added to the bag via the side addition port, using a peristaltic pump. Buffer solution (2 liters) was added via the same addition line to adjust the pH to the desired value. The temperature of the mixture was adjusted to 20 °C during slow mixing over approximately 1 hour. A solution of the highly toxic linker-payload (LP) in DMSO (about 200 mL) was slowly pumped peristaltically via a dip tube fitted on the top TC 1" ½ port while agitation was at 400 rpm, causing the temperature to rise to approximately 22–23 °C. The bottle, tubing and dip tube were rinsed with DMSO (about 100 mL) which was also pumped into the vessel. After an extended reaction time at 22 °C and 200 rpm agitation rate, the reaction mixture was sampled through the needle-free sampling port and the bag was drained via the bottom outlet tubing.

Assessment

Performance of the tested reaction in the Mobius® ADC reactor was comparable to the execution of the reaction at a 2 L scale in glass reactors. All the quality indicators tested were in acceptable ranges in comparison with the specifications.

Summary

Customer A concluded that the evaluation of the ADC reactor was a success. The use of the system and the installation of the single-use assembly were effortless, and the mixing proved suitable for the type of chemistry (bioconjugation) performed.

Customer B

Customer B performed some tests as well on the 500 L system. The characteristics of the 500 L reactor are described in Table 2.

Working volumes	50–500 L
Minimum volume for sampling and sensors	92 L
Mixing speed range	0–250 rpm
Operating Temperature Range	4 to 60 ºC

Table 2.

500 L reactor specifications.

Installation

Customer B found the bag installation to be straightforward, the different tubing lengths were considered appropriate, and they valued the inclusion of the tube holders for the vent filter tubing and dip tubes. Due to internal reasons, the load cells were not used but the unit fit well on their usual floor balance for weighing liquids by differential mass.

Worst-case working volume evaluation

While working volumes can range from 50 L to 500 L, the mixer underwent testing in a worst-case scenario with an initial volume of 50 L and a final volume of 170 L. This scale was imposed by the quantity of starting materials available for the test, but it also represented approximate volumes for the customer's typical production in R&D. At 50 L, for the first analytical control, the solution could be stirred, but the conductivity and pH electrodes, as well as the needle free sampling port, were not immersed. Later in the process, at the next analytical control, the volume increased to approximately 90 liters, allowing for sample collection for controls. The low volume in the bag resulted in moderate heat exchange. To cool the contents, a delta of 10 °C between the jacket and the content yielded a cooling rate of approximately -1 °C every 10 minutes.

mAb addition port evaluation

The mAb was introduced through the side port, flowing smoothly down the wall of the bag. Ordinarily, the flow rate is maintained at 1 L per minute; however, for this test under worst-case scenarios, it was increased to 3 L per minute. This increase did not result in excessive foaming, thus confirming that the standard flow rate of 1 L per minute is sufficiently cautious to virtually eliminate foam formation. Following this, buffer solutions were added through the same line to flush any residual mAb into the reactor.

Mixing speeds

While the speed range of the 500 L reactor reaches up to 250 rpm; given the minimal working volumes involved, the conjugation process predominantly utilized lower agitation speeds. Throughout the majority of this process, the speed was set at 40 rpm; although it was increased to 90 rpm to accommodate the addition of exothermic solutions. During the critical holding periods necessary for the chemical reactions to occur, the mixer was temporarily stopped.

Execution of an example coupling reaction

In this study, Customer B performed a stochastic reaction for the coupling between the monoclonal antibody and a linker-payload type molecule (LP).

The following table (Table 3) summarizes the analytical results comparing their historical data generated in their standard system (stainless steel reactor) with the one run performed in the Mobius® ADC reactor (therefore without optimization).

		Stainless Steel System	Mobius® ADC Reactor
Fragmentation	H2L2 (%)	89.2	91.5
	H2L (%)	7.4	5.7
	Σ fragments (%) (except H2L)	3.4	2.7
Charge Variant	Σ Low Isoform (%)	9.6	8.3
	Σ Main isoform (%)	81.8	81.6
	Σ High Isoform (%)	8.6	10.0
DAR* / Monomer purity	DAR	4.3	3.9
	Monomer %	97.3	96.7
	Σ HMW %	2.0	2.5

* The Drug to Antibody Ratio (DAR) represents the average number of drugs linked to each antibody. It stands as a critical property for assessing ADC quality, given its substantial impact on ADC efficacy.

Table 3.

Stochastic reaction analytical results.

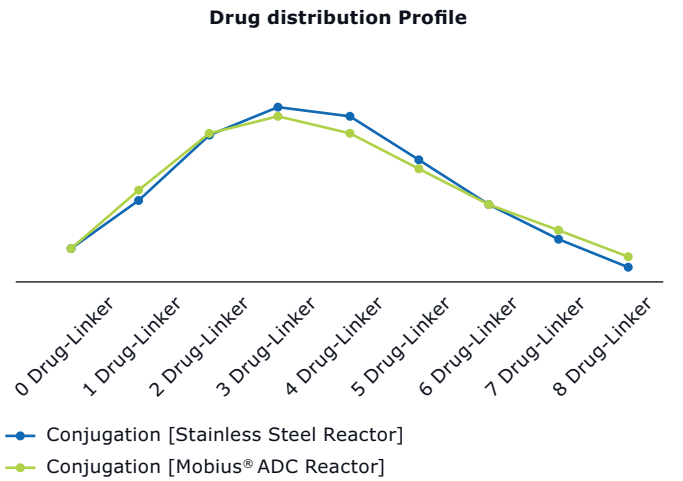


Figure 8.

Drug distribution profile.

Although a few minor differences were observed, they did not raise the customer´s concerns regarding the product or process under study. Their overall feedback confirmed that the test was deemed successful.

Summary

As the clinical pipeline for Antibody-Drug Conjugates continues to expand, the industry faces the challenge of providing manufacturers with safer and more efficient production methods. In response, we have developed a range of single-use ADC reactors (10 L, 100 L, and 500 L) that stand out from conventional mixing systems. The Mobius® ADC Reactor features solvent-compatible components, dip tube lines for solvent addition, and a dedicated monoclonal antibody side addition line. This innovation led to collaborations with two external customers, who were given the opportunity to test the 10 L and the 500 L reactors at their respective facilities, using their proprietary processes. The feedback from these trials was positive, with both customers considering their tests successful.

References

1. Application Note: Evaluation of Extractables and Physical Compatibility of the Mobius® ADC Reactor Single-Use Components for ADC Manufacturing MK_AN13726EN.
2. Mobius® ADC Reactor Datasheet MK_DS13685EN.

For additional information, please visit **www.SigmaAldrich.com/ADC-reactor**
To place an order or receive technical assistance, please visit **www.SigmaAldrich.com/contactAF**

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