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Application Note

Evaluation of Extractables and Physical Compatibility of the Mobius® ADC Reactor Single-Use Components for ADC Manufacturing.

Antibody-drug conjugates consist of a highly cytotoxic and potent active pharmaceutical ingredient (API) payload linked to a monoclonal antibody (mAb) capable of targeting a surface antigen on cancer cells. Conjugation of the mAb to the small molecule via the linker requires safe handling and containment due to the toxic nature of the payload. When used for this conjugation step, the single-use Mobius® ADC Reactor not only increases operator safety and process efficiency, scalability, reproducibility, and flexibility, but also decreases the risk of contamination.

The conjugation step typically includes the use of organic solvents, and as such, it is important to evaluate compatibility with the reactor's single-use components. The studies described in this document evaluated the physical compatibility of single-use components in the Mobius® ADC Reactor and the level of extractables when exposed to dimethyl sulfoxide (DMSO) and dimethyl acetamide (DMAc).

Single-use Components Evaluated for Physical Compatibility and Extractables

All single-use components were evaluated for extractables and physical compatibility following exposure to 100% or 20% DMSO, 100% or 20% DMAc, and Milli-Q® water. Typical solvent concentrations of DMSO and DMAc typically do not exceed 20% during conjugation. The various resins used in the molded plastic components of the Mobius® ADC Reactor were evaluated as tensile bars (Table 1).

Table 1. Molded plastic components evaluated for extractables and compatibility as tensile bars made from the corresponding resins

Item	Description	Material	Model Solvents
1	**mAb Addition Port**	Polyethylene	100% DMAc, 100% DMSO, and Milli-Q® water
2	**Impeller Cup**	Polyethylene	100% DMAc, 100% DMSO, and Milli-Q® water
3	**TC Port**	Polyethylene	100% DMAc, 100% DMSO, and Milli-Q® water
4	**Impeller**	Polypropylene	100% DMAc, 100% DMSO, and Milli-Q® water
5	**Port Plate**	Polyolefin	100% DMAc, 100% DMSO, and Milli-Q® water
6	**TC Cap**	Polypropylene	100% DMAc, 100% DMSO, and Milli-Q® water
7	**EJ Female Luer Barbed Fitting**	Polypropylene	100% DMAc, 100% DMSO, and Milli-Q® water

Pre-treatment	
Gamma Irradiation Dose	40 – 65 kGy
Pre-flush	No pre-flushing prior to extraction
Time between Gamma and Extraction	< 8 weeks
Extraction Conditions	
Extraction Solvents	100% DMAc, 100% DMSO, and Milli-Q® water
Surface Area to Volume Ratio	1:1 cm^2 / mL
Temperature	40 °C
Duration	72 hours
Conditions	Orbital rotation (50 rpm)

Table 2. Single-use gaskets, tubing, and single-use film evaluated for extractables and compatibility

Item	Description	Material	Model Solvents
8	**Gasket**	Platinum Cured Silicone	100% DMAc, 100% DMSO, and Milli-Q® water
9	**Dip Tube**	Non-Print PharMed Tubing	100% DMAc, 100% DMSO, and Milli-Q® water
10	**Pharma 50 Tubing**	Platinum Cured Silicone	100% DMAc, 100% DMSO, and Milli-Q® water
11	**Pharma 65 Tubing**	Platinum Cured Silicone	100% DMAc, 20% DMAc, 100% DMSO, 20% DMSO, and Milli-Q® water
12	**Pharma 80 Tubing**	Platinum Cured Silicone	100% DMAc, 100% DMSO, and Milli-Q® water
13	**Ultimus® Film**	LDPE (Outer Layer) ULDPE (Inner Layer) EVOH (Gas Barrier) ULDPE (Inner Layer) Woven Nylon and EVA (Strength Layer) ULDPE (Fluid Contact Layer)	100% DMAC, 20% DMAc, 100% DMSO, 20% DMSO, and Milli-Q® water

Pre-treatment	
Gamma Irradiation Dose	40 – 65 kGy
Pre-flush	No pre-flushing prior to extraction
Time between Gamma and Extraction	< 8 weeks
Extraction Conditions	
Extraction Solvents	100% or 20% DMAc, 100% or 20% DMSO, and Milli-Q® water
Surface Area to Volume Ratio	Varies depending on tubing ID. 6 : 1 or 2 : 1 cm^2 / mL for Ultimus® Film
Temperature	40 °C
Duration	72 hours (24 Hours, 100% DMAc and 100% DMSO for Ultimus® Film)
Conditions	Orbital rotation (50 rpm)

Experimental Design

Tensile Bars and Tubing Compatibility

Type 1 tensile bars representing the materials used in molded components, along with 6” (15 cm) samples of tubing and 5” x 1” (12 x 2 cm) of the Ultimus® film, were evaluated for tensile strength. Each sample was secured in pneumatic tensile grips and stretched using an Instron machine at a speed of 2” (5 cm) per minute. The percent relative difference between control (gamma irradiated, without solvent exposure) and stretched materials was plotted for each material.

Silicone Gasket Compressibility

A dynamic mechanical analyzer (DMA) instrument was used to assess the compressibility of post-gamma irradiated gasket samples under different solvent extraction conditions. During testing, the gasket underwent a 2% compression relative to its original thickness; the force required to achieve this deformation was recorded and plotted.

Extractables Testing

Analysis of volatile and semi-volatile organic compounds in the 100% DMAc and 100% DMSO extracts was performed using direct inject gas chromatography-mass spectrometry (DI-GC/MS). DI-GC/MS analysis was not performed on the water, 20% DMAc, and 20% DMSO extracts.

Reversed phase HPLC (RP-HPLC) was used to detect non-volatile organic substances in the extraction solution. Absorbance at UV 214 nm was used for detection of extractables.

Physical Compatibility and Extractables Results

Tensile Bars and Tubing Compatibility

None of the post-gamma irradiated tensile bars and tubing material (Figure 1), resins (Figure 2), or Ultimus® Film (Figure 4) samples displayed a notable decrease in measured tensile strength compared to the respective controls. These results demonstrated compatibility of the materials with Milli-Q® water, 100% DMAC, and 100% DMSO solvents under extraction conditions.

Figure 1. Relative change in tensile strength of resins used in single-use components following solvent exposure.

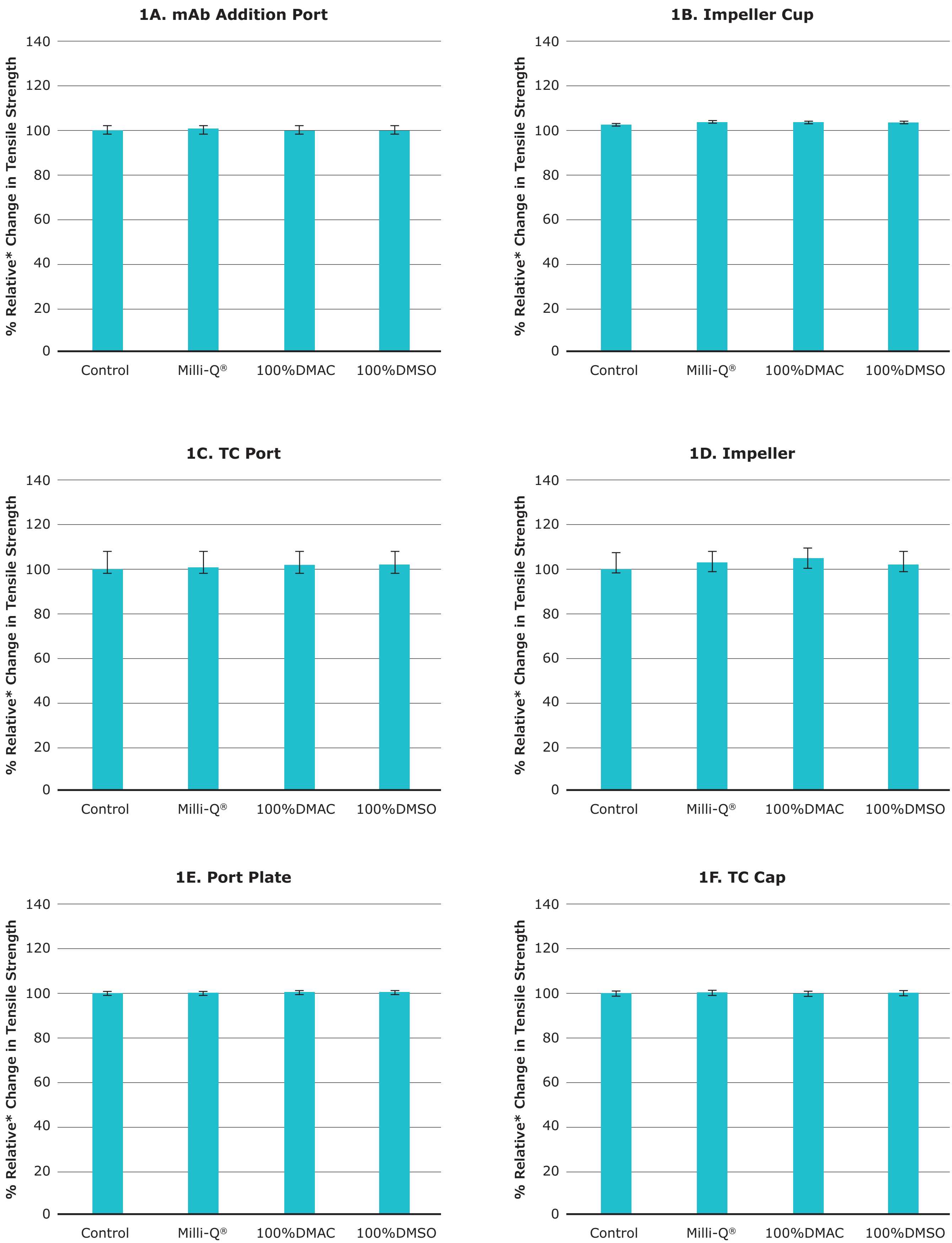


Figure 2. Relative change in tensile strength of resins used in single-use components following solvent exposure.

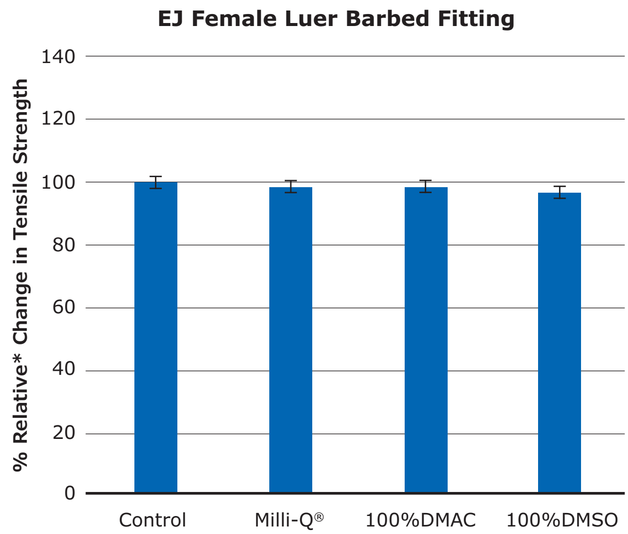
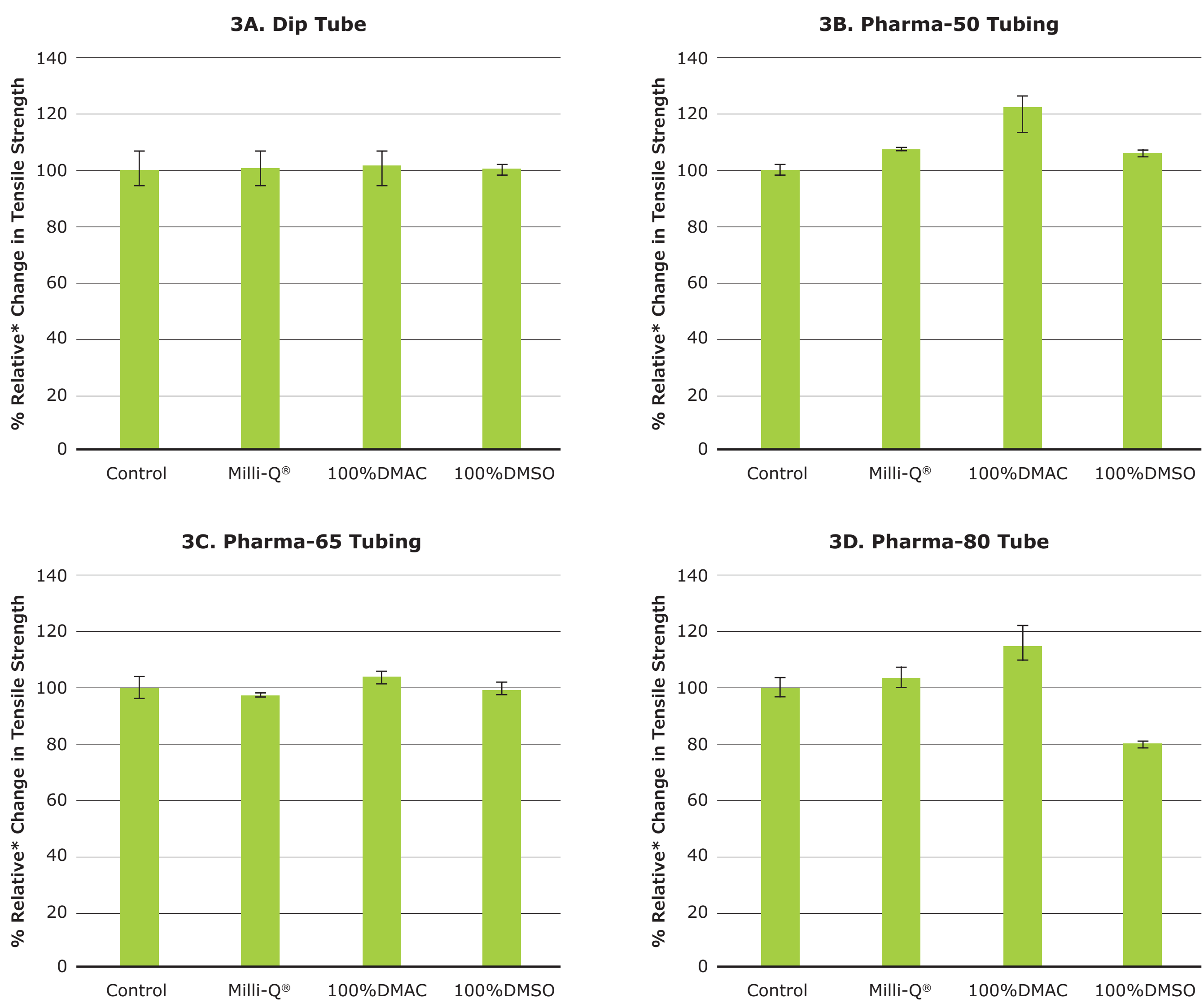


Figure 3. Relative change in tensile strength of tubing following solvent exposure



None of the post-gamma irradiated tensile bars with Ultimus® Film samples (Figure 4) displayed a decrease in measured tensile strength compared to the respective controls.

As shown in Figure 5, none of the post-gamma irradiated gasket samples displayed a notable decrease in compression force compared to their respective controls. These results demonstrated compatibility of the gasket samples with Milli-Q® water, 100% DMAC, and 100% DMSO solvents under extraction conditions.

Conjugation being followed by further purification steps such as tangential flow filtration and typically a buffer exchange, additional reduction of leachable components is expected.

Figure 4. Relative change in tensile strength of Ultimus® Film following solvent exposure

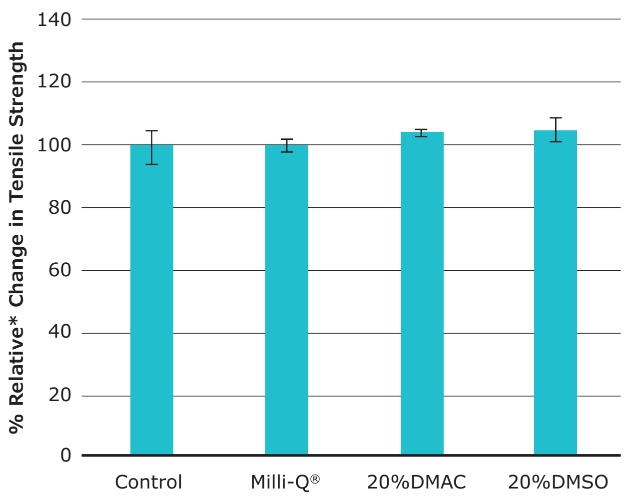
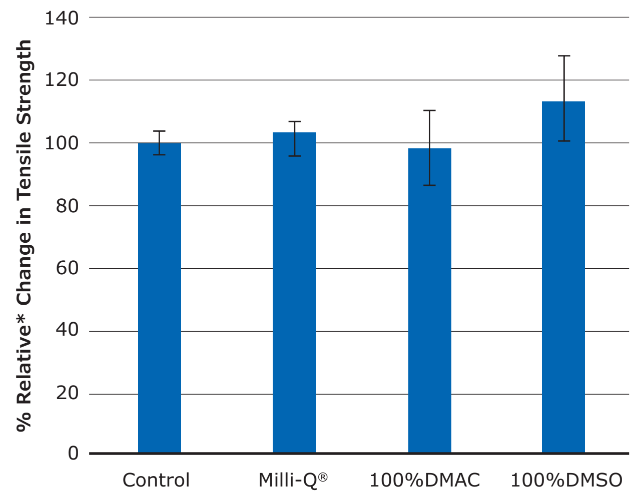


Figure 5. Relative change in compressibility of gasket samples following solvent exposure



Extractables

The following tables summarize results of the extractables studies. As shown by RP-HPLC and DI-GC/MS, the resins had excellent resistance when exposed to 100% DMAc and DMSO at 40°C for up to 72 hours with no observed visual or functional defects (Table 3). Similar results were obtained for gaskets, tubing, and Ultimus® Film with RP-HPLC (Table 4) and DI-GC/MS (Table 5).

Conjugation being followed by further purification steps such as tangential flow filtration and typically a buffer exchange, additional reduction of leachable components is expected.

Table 3. Detection of extractables from resins exposed to DMAc and DMSO using RP-HPLC and DI-GC/MS

Item	Material	HPLC Results in µg /cm²		DI-GC/MS Results in µg /cm²	
		100% DMAc	100% DMSO	100% DMAc	100% DMSO
	Reporting Limit	100 µg /cm²		0.5 µg /cm²	
1	**mAb Addition Port**	Non-Detected	Non-Detected	1,3-Di-tert-butylbenzene (1.8), Alkanes (1.0-2.4)	1,3-Di-tert-butylbenzene (0.9), Alkanes (0.6-0.9)
2	**Impeller Cup**	Non-Detected	Non-Detected	Non-Detected	Non-Detected
3	**TC Port**	Non-Detected	Non-Detected	Alkanes (0.6-1.5)	Alkanes (0.7)
4	**Impeller**	Non-Detected	Non-Detected	Unknown (0.8)	Non-Detected
5	**Port Plate**	Non-Detected	Non-Detected	1,3-Di-tert-butylbenzene (0.6)	1,3-Di-tert-butylbenzene (0.7)
6	**TC Cap**	Non-Detected	Non-Detected	Non-Detected	Non-Detected
7	**EJ Female Luer Barbed Fitting**	Non-Detected	Non-Detected	Non-Detected	Non-Detected

Table 4. Detection of extractables from gaskets, tubing, and Ultimus® Film exposed to DMAc and DMSO using RP-HPLC

Item	Material	DMAc (%)	Reporting Limit	HPLC Results	DMSO (%)	Reporting Limit	HPLC Results
8	**Gasket**	100	1000 µg / device	Non-Detected	100	1000 µg / device	Non-Detected
9	**Dip Tube**	100	70 µg /cm²	Non-Detected	100	70 µg /	Non-Detected
10	**Pharma 50 Tubing**	100	16 µg /cm²	Non-Detected	100	16 µg /	Non-Detected
11	**Pharma 65 Tubing**	100	7.5 µg /cm²	Non-Detected	100	7.5 µg /	Non-Detected
12	**Pharma 80 Tubing**	100	48 µg /cm²	Non-Detected	100	48 µg /	Non-Detected
13	**Ultimus® Film**	100	50 µg /cm²	Non-Detected	100	50 µg /	Non-Detected
14	**Ultimus® Film**	20	3.3 µg /cm²	Non-Detected	20	3.3 µg /	Non-Detected

Table 5. Detection of extractables from gaskets, tubing, and Ultimus® Film exposed to DMAc and DMSO using RP-HPLC

Item	Material	DMAc (%)	Reporting Limit	DI-GC/MS Results	DMSO (%)	Reporting Limit	DI-GC/MS Results
8	**Gasket**	100	5 µg /device	1,3-Di-tert-butylbenzene (21), Bis(2-ethylhexyl) adipate (13), siloxanes (7-82)	100	5 µg /device	1,3-Di-tert-butylbenzene (15), Dimethyl phthalate (7), Bis(2-ethylhexyl) adipate (12)
9	**Dip Tube**	100	33 µg /cm²	Non-Detected	100	0.4 µg /cm²	2,4-Di-tert-butylphenol (0.5), unknown (0.4)
10	**Pharma 50 Tubing**	100	0.08 µg /cm²	Siloxanes (0.09-103)	100	0.08 µg /cm²	Siloxanes (0.08-4.7)
11	**Pharma 65 Tubing**	100	0.04 µg /cm²	Mainly siloxanes (0.04-30)	100	0.04 µg /cm²	Mainly siloxanes (0.04-2.1)
12	**Pharma 80 Tubing**	100	0.2 µg /cm²	Mainly siloxanes (0.2-355)	100	0.2 µg /cm²	Mainly siloxanes (0.3-18)
13	**Ultimus® Film**	100	0.3 µg /cm²	Mainly alkanes and alkenes (0.4-0.8)	100	0.3 µg /cm²	Non-Detected

Note: Mineral oil was found in 100% DMAc extracts

Conclusion

Single-use technologies offer many benefits for production of ADCs including increased operator safety, process efficiency, scalability, reproducibility, and flexibility. As with all single-use systems, understanding solvent compatibility is essential for a successful and safe implementation. As demonstrated by the results described in this document, 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.

For additional information

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