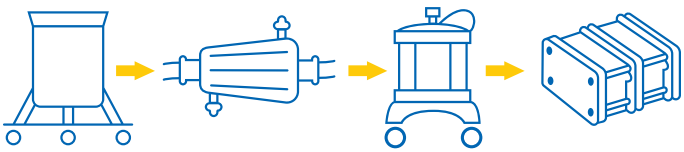
Millipore®
Preparation, Separation,
Filtration & Monitoring Products

Application Note

Compatibility of a Mobius® Single-use Solution for ADC Processing

Introduction

Antibody Drug Conjugates (ADCs) are a class of bio-molecules that has seen rapid growth as an oncology therapeutic. ADCs are comprised of three parts: monoclonal antibody (mAb), linker, and cytotoxic payload, where each component plays a role in therapeutic efficacy. Following linker/payload addition to the mAb, additional downstream unit operations are needed to purify the material. These often include chromatography, tangential flow filtration (TFF) and sterile filtration as shown below.



The small molecule linker/payload is a highly potent, toxic agent that requires special consideration for safe handling and containment during conjugation and downstream processing. To address these concerns, many ADC manufacturers are adopting single use technologies. The transition from traditional manufacturing in stainless steel or glass, to single-use poses challenges, as the conjugation step commonly utilizes aqueous organic solvents. Understanding component compatibility during conjugation and purification in the presence of these solvents is needed to enable adoption of single-use technologies in ADC processing.

This study evaluated physical compatibility and extractables results of a proposed single-use solution for conjugation, purification and sterile filtration of ADCs in the presence of Dimethyl Sulfoxide (DMSO) and Dimethyl Acetamide (DMAc), including a Mobius® Mixer for conjugation, a Millipore Express® SHC capsule for sterile filtration prior to purification, a Mobius® Flexready system with Smart Flexware® configured for chromatography or tangential flow filtration, Lynx® CDR and Lynx® S2S sterile connectors. Mobius® systems described in this study utilize a common film, therefore compatibility and extractables results are representative across the systems. Typical aqueous solvent concentrations of DMSO or DMAc do not exceed 20% during conjugation and these concentrations were utilized for much of the evaluation reported here. Film compatibility with 100% DMSO and DMAc was also evaluated to consider inadvertent contact during addition of linker/payload dissolved in 100% DMSO or 100% DMAc.

Results indicate excellent resistance to these solvents over 24 hours exposure at temperatures up to 40°C, with no observed visual or functional defects. Extractables testing for 38 metals found no elements that are to be considered in a risk assessment as outlined in ICH Q3D Guideline for Elemental Impurities and USP <232> Elemental Impurities. Additional testing for volatile, semi-volatile, and non-volatile organic compounds did not identify any compound at a concentration greater than 0.05 µg/mL in the Smart Flexware® or sterile connectors. Very few organic compounds were detected in the Millipore Express® SHC capsule, and levels would fall below 0.10 µg/mL in a filtered pool even at a fairly low filter throughput of 150 L/m^2. In addition, since the tangential-flow filtration operation includes a diafiltration step to exchange buffer, additional removal of leachable components is expected to be achieved.[1] In-depth testing methodology and results can be found in this application note.

MERCK

Mobius® mixer

PureFlex™ film is utilized in both Mobius® mixers and FlexReady systems. Analysis for 20% DMSO and 20% DMAc exposure can be found in the FlexReady system discussion later in the document. In this section, we describe the design and results of 100% solvent exposure to the film.

100% Solvent exposure to PureFlex™ film for Mobius® systems

Test design

PureFlex™ bags were created, gamma irradiated and evaluated for compatibility with 100% DMSO, 100% DMAc, or RO (Reverse Osmosis) water. Two hundred-forty milliliters of the appropriate solvent were added to the bags and placed on an orbital shaker at 50 rpm for 4 or 24 hours at 40 °C.

Compatibility evaluation

Following solvent exposure, bags were rinsed and tested for tensile strength by evaluating both bag seam strength and film flexibility. Testing was conducted using an INSTRON Universal testing machine. Test method ASTM D882-12 Standard Test Method for Tensile Properties of Thin Plastic Sheeting was used for the analysis. Strips of film were die-stamped from flat sections and from areas across the seams of the test bags allowing for a 180 degree pull test on the seam. These samples were tensile tested and compared against two types of control samples – either virgin film or film exposed to Milli-Q® water only. Film samples were also visually inspected to detect any appearance changes that occurred.

Results

Exposure to 100% solvent (DMSO or DMAc) for up to 24 hours at 40 °C had no effect on bag seam strength and an increase in film elongation at break. Since the bag will be supported in a carrier during use, it is not expected that the increase in film elongation will have any impact on successful processing. No visual changes were observed.

Sterile Filter

To evaluate compatibility with standard ADC solvents, Millipore Express® SHC membrane was evaluated first in membrane disc format followed by evaluation of a single use filter capsule.

Compatibility test design and evaluation

Millipore Express® SHC membrane discs

Millipore Express® SHC membrane discs (47 mm) were installed into holders and wet with water by applying vacuum of 10 psig. Water flow rate through the membrane was measured followed by bubble point measurements. The discs were dried and installed into stainless steel holders connected to a peristaltic pump. Recirculation of 20% DMSO (Dimethyl Sulfoxide Emprove® Expert Ph Eur. USP) or 20% DMAc at 40 °C ±2 °C was performed at ~ 10 psi to ensure complete wetting. The discs were then soaked for ≥24 hours at 40 °C ±2 °C. After exposure, product bubble point was measured and then the discs were visually inspected for signs of swelling, shredding, change of color, dissolution and any other apparent physical changes. Finally, the discs were re-installed into clean holders and flushed with water using vacuum at 10 psig. Water flow rate and bubble point measurements were repeated post-solvent exposure. The results of pre-use, solvent, and post-solvent exposure were compared, as summarized in Table 1.

Table 1. Comparison of pre- and post-solvent exposure water flow rate and bubble point on Millipore Express® 47 mm discs

20% DMSO					
Sample	Initial Water Flow Rate (mL/min)	Initial Water Bubble Point (psi)	Product Bubble Point (psi)	Final Water Flow Rate (mL/min)	Final Water Bubble Point (psi)
1	318.3	82.5	75.6	301.4	81.5
2	324.2	80.9	73.8	308.8	79.6
3	318.3	81.6	75.5	302.2	82.7

20% DMAc					
Sample	Initial Water Flow Rate (mL/min)	Initial Water Bubble Point (psi)	Product Bubble Point (psi)	Final Water Flow Rate (mL/min)	Final Water Bubble Point (psi)
1	318.2	86.2	67.7	334.9	85.8
2	322.9	82.6	64.2	341.8	80.8
3	310.5	86.5	68.3	313.0	85.6

Opticap® XL capsules with Millipore Express® SHC membrane

A 3" Opticap® XL Millipore Express® SHC (KHGEG03TT3) capsule was connected to a peristaltic pump and 20% DMSO (Dimethyl Sulfoxide Emprove® Expert Ph Eur. USP) or 20% DMAc was recirculated at a flow rate of ≥1.4 L/min at 40 °C ±2 °C to ensure complete filter wetting. The capsule was then soaked for ≥24 hours at 40 °C ±2 °C.

After solvent exposure, capsules were evaluated for product bubble point as determined from membrane disc testing above, and visually inspected for signs of swelling, shredding, change of color, dissolution or other physical changes.

Millipore Express® membranes exhibit excellent compatibility with 20% DMSO or 20% DMAc as shown by comparison of initial and final water bubble point and water flow rates through the membrane (Table 1). In addition, the capsule passed bubble point evaluation.

Visual examination of 47 mm discs and capsules post exposure to DMSO did not indicate any significant changes in color, shredding, swelling or other observable physical change, while after exposure to DMAc a slight swelling of the membrane was observed but this had no impact on membrane or device integrity test results. No other visible physical changes were observed with DMAc exposure.

Extractables test design and evaluation

Millipore Express® SHC capsules exposure to solvent for extractables evaluation

Without any pre-use flushing, 5" Opticap® XL capsule with Millipore Express® SHC (KHGES05TT1) were filled with 20% DMSO (Dimethyl Sulfoxide for molecular biology, Sigma-Aldrich, D8418), 20% DMAc (N,N – dimethyl acetamide, anhydrous 99.8%, Sigma-Aldrich, 271012), or RO water, placed into an oven set to 40 °C, and held for 24 hours. Samples for extractables analysis were collected at 0.5, 4, 8 and 24- hour time points and analyzed for volatile, semi-volatile and non-volatile compounds.

Volatile and semi-volatile organic analysis

Volatile and Semi-volatile organic compounds were evaluated with HS-GC/MS (headspace gas chromatography-mass spectrometry) and DI-GC/MS (direct inject gas chromatography-mass spectrometry), respectively. All peaks with a response greater than 0.1 µg/mL that were detected in the injection sample at a level 1.5x or higher than in the associated control samples were reported as extractables. Injection samples were prepared by concentrating test samples 20x prior to GC-MS analysis. Therefore, detection sensitivity as related back to the test samples was 0.005 µg/mL.

Non-volatile organic analysis

Non-volatile organic compounds were evaluated with LC-MS (liquid chromatography-mass spectrometry) using electrospray ionization positive and negative polarity modes (ESI ±). Post-acquisition, chromatograms were assessed for extractable peaks and the collection of associated mass spectra. Reference standards were analyzed in attempt to identify unknown peaks. Limit of detection for the analysis was 0.05 µg/mL.

Extractables results

The results of extractables are reported in milligrams of extractables per 5" capsule [mg/capsule] and were obtained by multiplying the assay result value [µg/mL] by the average volume of solvent added to the capsules for each solvent [mL], which ranged from 756 mL to 785 mL.

Volatile organic analysis

HS-GC/MS analysis for volatile organic compounds identified one compound, isopropyl alcohol, in capsules exposed to water and capsules exposed to 20% DMSO. No compounds were detected for capsules exposed to 20% DMAc. Total amounts per capsule are shown in Figure 1.

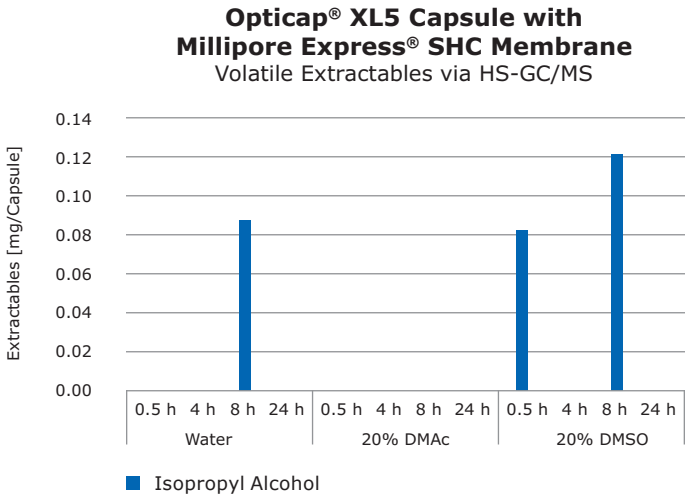


Figure 1: Volatile extractables for Opticap® XL5 Capsules with Millipore Express® SHC membrane

Semi-volatile organic analysis

DI-GC/MS analysis for semi-volatile organic compounds (SVOC) identified three compounds in capsules exposed to water (diphenyl sulfone, N-methyl-pyrrolidinone (NMP), hexylene glycol) and one compound (4-metoxy-3-penten-2-one) in capsules exposed to 20% DMAc. Diphenyl sulfone is a solvent used to manufacture PES, NMP and hexylene glycol are solvents used to make the membrane. No compounds were detected for capsules exposed to 20% DMSO. Total amounts per capsule for each identified compound are shown in Figure 2.

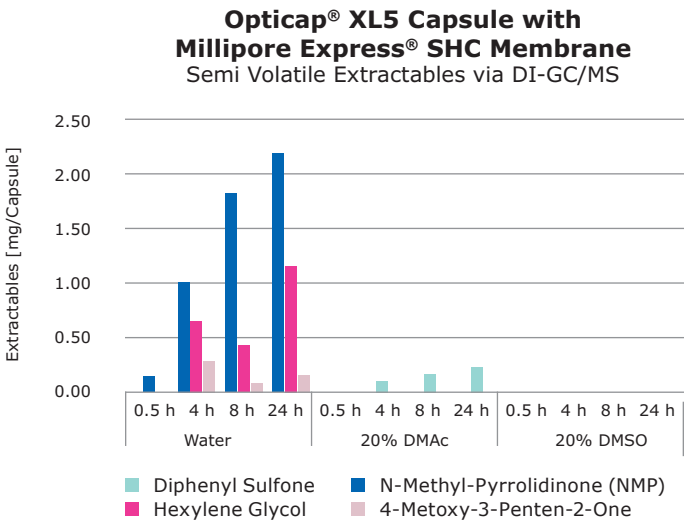


Figure 2: Semi-volatile extractables for Opticap® XL5 Capsules with Millipore Express® SHC membrane

Non-volatile organic analysis

HPLC-DAD/MS analysis for non-volatile organic compounds identified one compound, diphenyl sulfone, in capsules exposed to 20% DMAc and in capsules exposed to 20% DMSO. One compound, NMP was also detected for capsules exposed to water. Both compounds were also identified in the SVOC testing. Total amounts per capsule for each identified compound are shown in Figure 3.

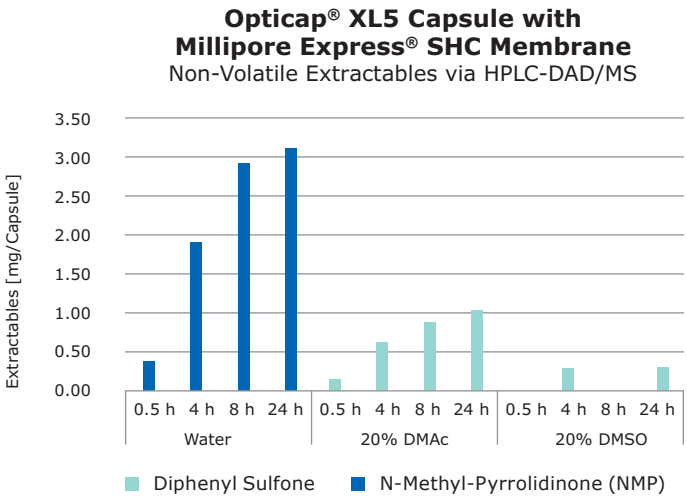


Figure 3: Non-volatile extractables for Opticap® XL5 Capsules with Millipore Express® SHC membrane

The identified extractables from Opticap® XL5 Capsules with Millipore Express® SHC membrane are components related to either the membrane manufacturing process or gamma irradiation. The concentration of these compounds in a filtered pool would be determined by dividing the total amount extracted per capsule, as shown in the graphs, by the volume filtered through the capsule during processing. As filter throughput increases, the extractable concentration in the pool would decrease. In addition, any pre-use flushing of the filter would further reduce the extractable concentration in the pool. The results in this study demonstrate that, even with no pre-use flushing, if a relatively low filter throughput of 150 L/m^2 is achieved, all compounds would be below 0.10 µg/mL concentration. In addition, some level of clearance of leachables can be achieved during a subsequent ultrafiltration/diafiltration step, in which leachables pass through the membrane of the filtration device while the product is retained[1].

Bacterial retention test design and evaluation

Bacterial retention capabilities of Opticap® XL150 capsule filters with Millipore Express® SHC membrane were evaluated after exposure to 20% dimethyl sulfoxide (DMSO), 20% dimethyl acetamide (DMAc) and Milli-Q® water (control). Recirculation flow loops for the DMSO and DMAc exposures, unique to each solvent, contained two Opticap® XL150 Capsule filters with Millipore Express® SHC membrane. The recirculation flow loop for the water control exposure contained one Opticap® XL150 Capsule filter with Millipore Express® SHC membrane. All flow loops were gamma irradiated at 40-60 kGy prior to testing. Testing consisted of recirculating 600 mL of solvent in each flow loop for 20 minutes at a flow rate of ≤ 1.5 L/min and subsequent incubation in a 40°C oven for 24 hours. Afterwards, the flow loops were emptied and flushed with Milli-Q® water. Capsules were disconnected from the loops and submitted for bacterial retention testing.

Each device was challenged with *Brevundimonas diminuta* (ATCC® 19146™) which was prepared using a two-step culture method based on the ASTM F838 standardized methodology[2] for *B. diminuta* culture for qualifying sterilizing-grade membrane by retention testing. The retention test was performed using a forward pressure of 30 psi at ambient temperature. The challenge feed was prepared to meet the required greater than 10^7 CFU (colony forming units) per cm^2 of effective filtration area. In addition, one new device, unexposed to solvent, was also challenged using the same method.

Complete retention of *B. diminuta* was observed in all Opticap® XL150 samples with Millipore Express® SHC membrane. Durapore® 0.45 µm filter, used as a positive control, demonstrated 1.7 LRV passage of *B. diminuta*, which is consistent with historical data. The results are summarized in Figure 4 and demonstrate that Opticap® XL150 capsule filters with Millipore Express® SHC membrane are fully retentive after exposure to 20% DMSO and 20% DMAc.

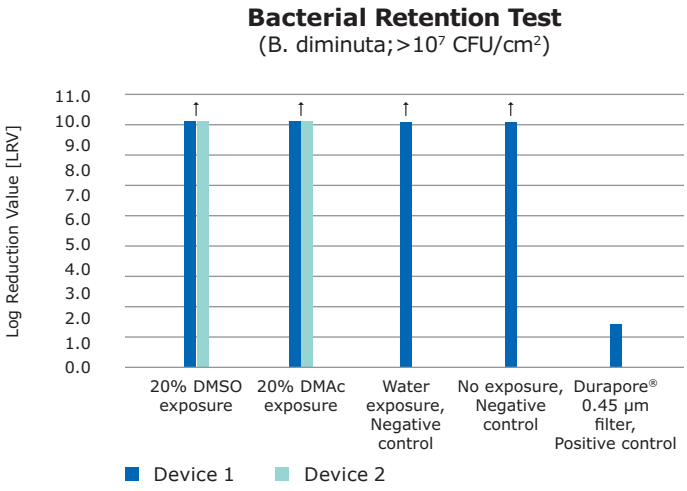


Figure 4: Bacterial Retention Testing Results for Opticap® XL150 Capsules with Millipore Express® SHC Membrane with and without exposure to Solvents (upward facing arrow indicates complete Retention)

Mobius® FlexReady Solution with Smart Flexware® and sterile connectors

Test design

Solvent exposure to Mobius® FlexReady Solution with Smart Flexware® assemblies for TFF

All single-use components of the Mobius® FlexReady Solution with Smart Flexware® Assemblies were evaluated for compatibility with 20% DMSO (Dimethyl Sulfoxide for molecular biology, Sigma-Aldrich, D8418), 20% DMAc (N,N–dimethylacetamide, anhydrous 99.8%, Sigma-Aldrich, 271012) and RO water. The wetted flow path included a feed container, pump head, Smart Flexware® assembly, retentate sampling port, as well as a sensor flow cell. All wetted materials were single-use and gamma irradiated. Cassette liners and filtration devices were excluded from the setup; instead, a segment of Dow Corning® Pharma-80 tubing was used to create a bypass between the feed and retentate ports of the Flexware® Assembly and the sensor flow cell was connected in this line. A schematic of the setup and a list of components used during recirculation is summarized in Figure 5 and Table 2.

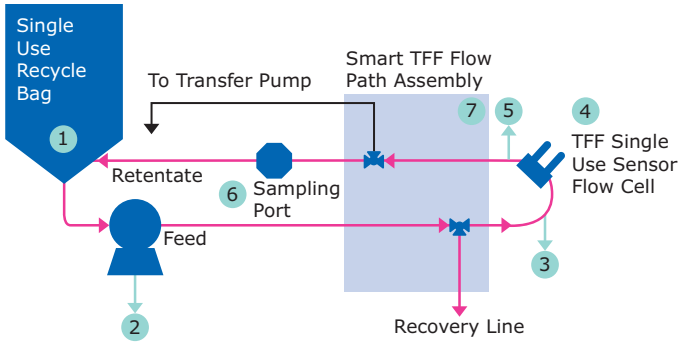


Figure 5: Schematic of the Test Setup used during solvent recirculation in the Mobius® FlexReady Solution with Smart Flexware® Assemblies for TFF

Item	Part Description
1	100L Feed Container Assembly
2	Single Use Feed Pump Assembly
3, 5	Dow Corning® Pharma-80 Tubing
4	Single Use Sensor Assembly
6	Sampling Port Assembly
7	Smart Flexware® Assembly

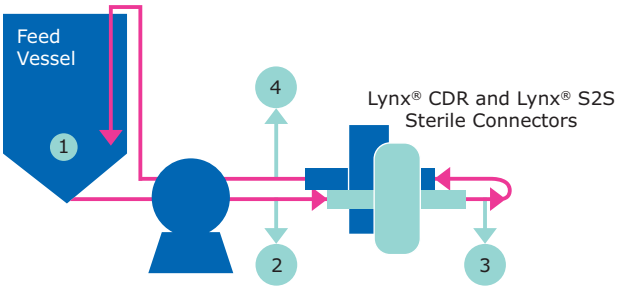
Table 2: List of Components used during solvent recirculation in the Mobius® FlexReady Solution with Smart Flexware® Assemblies for TFF

Six liters of solvent was recirculated through the system for 24 hours at room temperature, low flow rate (approximately 1.14 L/min) and low pressure (< 0.3 bar). Samples for extractables analysis were collected at pre-determined time points, with fresh solvent added after each sampling to maintain a constant recirculating volume. A new flow path assembly was installed for each solvent.

Solvent exposure to Lynx® CDR, Lynx® S2S and Colder AseptiQuik® G sterile connectors flow loops

Three types of sterile-to-sterile connectors were assembled into flow loops and evaluated for compatibility with 20% DMSO, 20% DMAc and RO water. One set of flow loops included one Lynx® CDR and one Lynx® S2S sterile connector, while the second set of flow loops included two Colder AseptiQuik® G sterile connectors. A unique flow loop was created for each solvent and was tested separately. A schematic of the setup and a list of components used during recirculation is summarized in Figure 6 and Table 3.

Lynx® Connectors Flow Loop



AseptiQuik® G Flow Loop

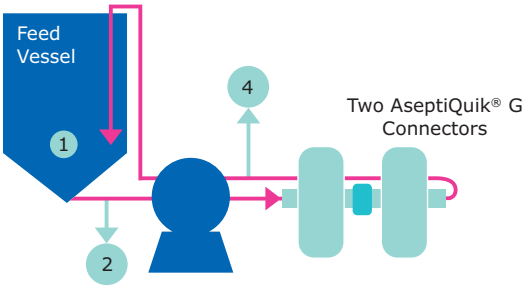


Figure 6: Schematic of Lynx® and AseptiQuik® G Connectors Setup used during solvent recirculation

Item	Description
1	SS Feed Vessel
2	Dow Corning® Pharma-65 Tubing
3	Dow Corning® Pharma-80 Tubing
4	Dow Corning® Pharma-50 Tubing

Table 3: List of Components used during Solvent Recirculation

Five hundred milliliters of solvent was recirculated through each of the flow loops for 24 hours at room temperature, low flow rate (< 1 L/min) and low pressure (< 0.3 bar). Samples for extractables analysis were collected as described in the previous section. A full list of materials of construction that were exposed to solvent in this study are shown in Table 4.

Material of Construction	Component
PureFlex™ Film	Feed container, Smart Flexware® assembly
Silicone	Tubing, Sanitary gaskets, O-rings (Lynx® S2S, Lynx® CDR), Colder MPX, Colder AseptiQuik® G
Polysulfone	Connectors (Colder MPX, Lynx® S2S)
Polypropylene	Adaptors, mixer impeller, disposable pump head, couplers
Polyolefin Elastomer	Feed container ports
HDPE	Retentate diverter, vortex breaker, sample port, low dead volume tee
EPDM Rubber	Mixer impeller retainer, disposable pump head, Single Use sensor flow cell
Santoprene	Disposable pump head
Polyphenylsulfone	Single Use Sensor Flow cell
Quartz	Single Use Sensor Flow cell
Epoxy	Single Use Sensor Flow cell
316 Stainless Steel, Electropolished and passivated	Lynx® CDR male spring
Glass filled Polysulfone	Lynx® CDR
Polycarbonate	Colder AseptiQuik® G connectors

Table 4: List of Materials of Construction exposed to Solvent.

Compatibility evaluation

Post-solvent exposure visual inspection

After 24-hour solvent recirculation and final sampling, solvent was drained, and all assemblies were flushed thoroughly with RO water. All single-use components from the TFF system as well as the connector loops were visually inspected for any physical and appearance changes resulting from solvent exposure.

Post-solvent exposure functionality testing

After visual inspection, the Lynx® CDR, Lynx® S2S and AseptiQuik® G connector assemblies were incorporated into the feed/retentate bypass loop of the Mobius® FlexReady Solution with Smart Flexware® Assemblies for TFF and the functionality of all assemblies was evaluated via Valve Bypass testing and Valve Cycling testing using room temperature water. These tests were developed to qualify that manufactured Flexware® Assemblies can achieve the maximum process claims for flow, pressure, duration, and valve cycles. While the testing is typically run on new assemblies, performing the evaluation after solvent exposure is a rigorous way to confirm whether the solvent has any detrimental impact on process capability.

Valve bypass testing

Two configurations, shown in Figure 7a and Figure 7b were tested; the first at a flow rate of 18 L/min and feed pump discharge pressure of 4 bar; the second at a flow rate of 9 L/min and feed pump discharge pressure of 2 bar. The duration of each test was 10 minutes. Sealing integrity of the valves within the Mobius® FlexReady solution clamshell as well as all components and connections external to the clamshell were inspected throughout and after the test.

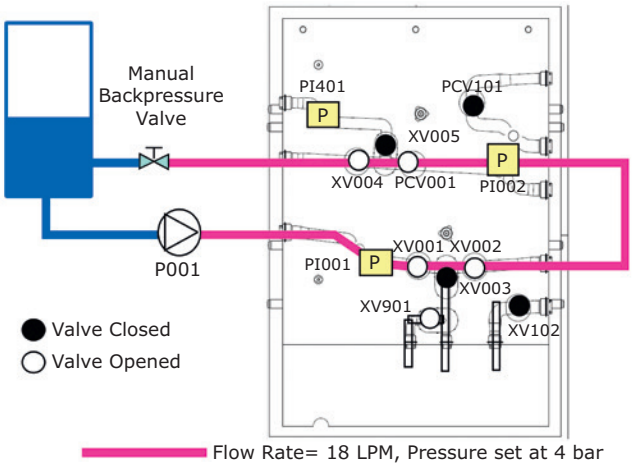


Figure 7a: Valve Bypass Testing, Configuration 1

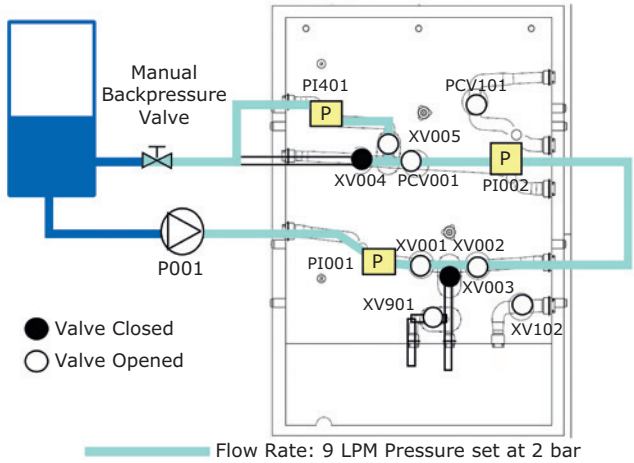


Figure 7b: Valve Bypass Testing, Configuration 2

Valve cycling testing

Valve cycling test was performed after valve bypass testing. The flow rate was set to 18 L/min and pressure was set to 4 bar in one section of the flowpath, 2 bar in another section of the flowpath. While these flow and pressure conditions were maintained, two valves were each subjected to 103 open/close cycles. Sealing integrity of the valves within the Mobius® FlexReady solution clamshell as well as all components and connections external to the clamshell were monitored throughout the test. After the valve cycling test was completed, the valve bypass testing was repeated to determine if sealing integrity was still maintained. The valve cycling test setup is shown in Figure 8.

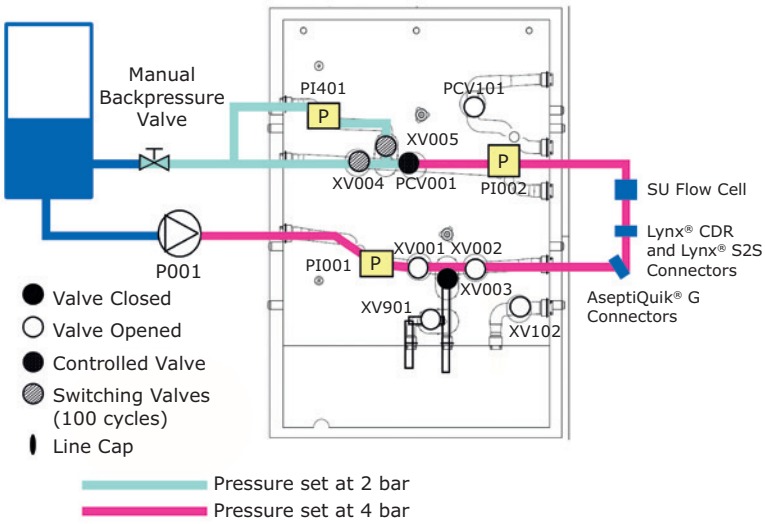


Figure 8: Valve Cycling Test Setup

Extractables testing

Samples were collected at pre-determined time points for extractables evaluation and the following test methods were used for analysis.

Metal analysis

Metals analysis was performed using ICP-OES (Inductively coupled plasma optical emission spectroscopy) for 38 metal elements. Metals analysis was performed only on water samples due to assay interference with DMSO and DMAc. Solvent is not expected to be more aggressive than water for metals extraction. Sample detection limit was in the range of 0.02 - 0.03 µg/mL.

Volatile and semi-volatile organic analysis

Volatile and Semi-Volatile Organic compound analysis were evaluated with HS-GC/MS (headspace gas chromatography-mass spectrometry) and DI-GC/MS (direct inject gas chromatography-mass spectrometry), respectively. All peaks with a response greater than 0.1 µg/mL that were detected in the injection sample at a level 1.5x or higher than in the associated control samples were reported as extractables. Injection samples were prepared by concentrating test samples 20x prior to GC-MS analysis. Therefore, detection sensitivity as related back to the test samples was 0.005 µg/mL.

Non-volatile organic analysis

Non-volatile organic compounds were evaluated with LC-MS (liquid chromatography-mass spectrometry) using electrospray ionization positive and negative polarity modes (ESI ±). Post-acquisition, chromatograms were assessed for extractable peaks and the collection of associated mass spectra. Reference standards were analyzed in attempt to identify unknown peaks. Limit of detection for the analysis was 0.05 µg/mL.

Results

Compatibility

Visual inspection of components

Visual inspection did not reveal any leaks throughout the 24-hour recirculation, and no changes in physical appearance of single-use components were detected as a result of 24-hour contact with solvents. No cracks, crazing, deformation, discoloration, or “stickiness” of components was detected.

Valve bypass testing

No leaks or bypass were observed in any part of the flow path. Flexware® assemblies for Mobius® sysytems and all connectors passed the test before and after the valve cycling test.

Valve cycling testing

No leaks or damage to any of the components were detected throughout the duration of the test.

Extractables

Extractables calculations

Since the Mobius® FlexReady Smart Flexware® Assemblies and the sterile connector flow loops incorporate many different components and materials of construction, the standard approach of basing extractables on exposed surface area was impractical. Instead, an aggregated approach was taken and the results of extractables are reported in micrograms of extractables per system [µg/system] and were obtained by multiplying the assay result value [µg/mL] by the system volume basis [mL] at the timepoint that the sample was collected. The method for determining system volume basis for each system is described below.

Mobius® FlexReady Solution with Smart Flexware® Assemblies System:
Total extractables for each assay, each solvent, and each timepoint are calculated based on the 6L that was recirculated in the system. No adjustments were made for sample volumes that were removed and replaced since they are small (approximately 3%) in comparison to the total recirculating volume.

Lynx® CDR and Lynx® S2S Connectors System:
Total extractables for each assay and each solvent are calculated based on the 0.5L that was recirculated plus the volume of additional solvent that was replenished after each timepoint sample. Sample replenish volume was 180 mL for the water extraction, for 4 sample timepoints. Sample replenish volume was 120 mL for the DMSO and DMAc extractions, for 3 sample timepoints. Table 5 shows the volume basis for each solvent and sample point.

AseptiQuik® G Connectors System:
Total extractables were calculated following the methods described in the section for Lynx® CDR and Lynx® S2S connectors.

Solvent	Volume Basis [mL]				
	0.5 H Sample	2 H Sample	4 H Sample	8 H Sample	24 H Sample
Water	500	680	860	1040	1220
20% DMSO	500	n/a	620	740	860
20% DMAc	500	n/a	620	740	860

Table 5: System volume basis for extractables reporting of Lynx® CDR, Lynx® S2S, and Aseptiquick® G connectors for each sampling point and solvent.

Metals analysis

Only traces of sodium and calcium were detected in the submitted samples. No metals identified are classified as elements to be considered in the risk assessment as outlined in ICH Q3D Guideline for Elemental Impurities and USP <232> Elemental Impurities.

Non-volatile organic analysis

No compounds were detected in any system or solvent.

Volatile organic analysis

HS-GC/MS analysis for volatile organic compounds indicated no extractable peaks in any system or solvent.

Semi-volatile organic analysis

DI-GC/MS analysis for semi-volatile organic compounds did not identify any peaks above 0.05 µg/mL. A few peaks were identified at a concentration of <0.05 µg/mL in the Mobius® FlexReady Solution with Smart Flexware® Assemblies, Lynx® CDR, Lynx® S2S and AseptiQuik® G connectors exposed to water, 20% DMSO and 20% DMAc, as shown below in Figures 9 through 14.

Mobius® FlexReady Solution with Smart Flexware® assemblies for TFF and connectors exposed to water

As seen in Figure 9, 2,4 Di-tert-butylphenol (degradant of an antioxidant), D5 siloxane (extractables from silicone) and 2-phenyl-2-propanol (reported as cumyl alcohol) were detected in samples collected from the Mobius® Smart Flexware® Assemblies. Figure 10 shows that several different siloxanes were detected in samples collected from the AseptiQuik® G connectors exposed to water for 24 hours. No compounds were detected in samples collected from the Lynx® connectors. All individual component levels were below 0.05 µg/mL

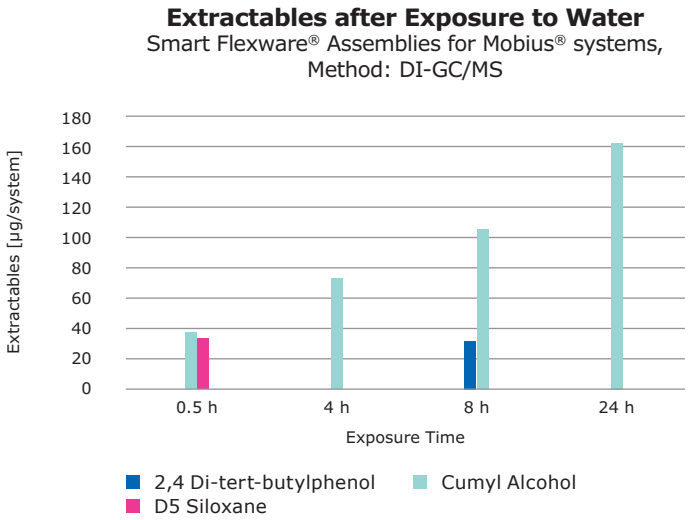


Figure 9: Extractables for Smart Flexware® Assemblies for Mobius® systems, exposed to Water

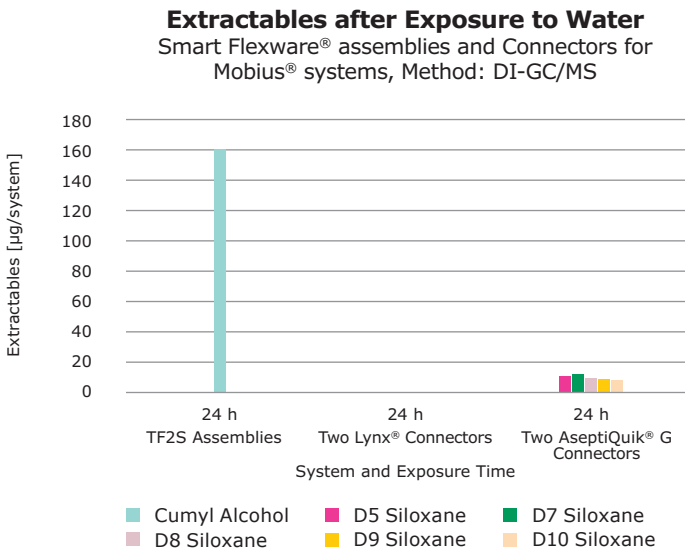


Figure 10: Extractables for Smart Flexware® Assemblies and Connectors for Mobius® systems, exposed to Water

Mobius® FlexReady solution with Smart Flexware® Assemblies for TFF and connectors exposed to 20% DMSO

Figure 11 shows that only one extractable compound, 2,4 di-tert-butylphenol was detected in samples collected from Mobius® Smart Flexware® Assemblies after exposure to 20% DMSO. At the 24-hour time point, the compound was below the assay limit of detection. D6 Siloxane was detected in samples collected from the Lynx® and AseptiQuik® G connectors at 24 hours, as shown in Figure 12. All individual component levels were below 0.05 µg/mL.

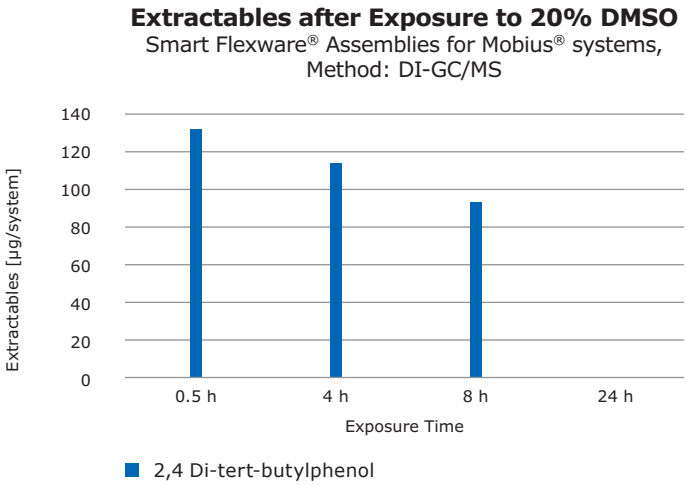


Figure 11: Extractables for Smart Flexware® Assemblies for Mobius® systems exposed to 20% DMSO

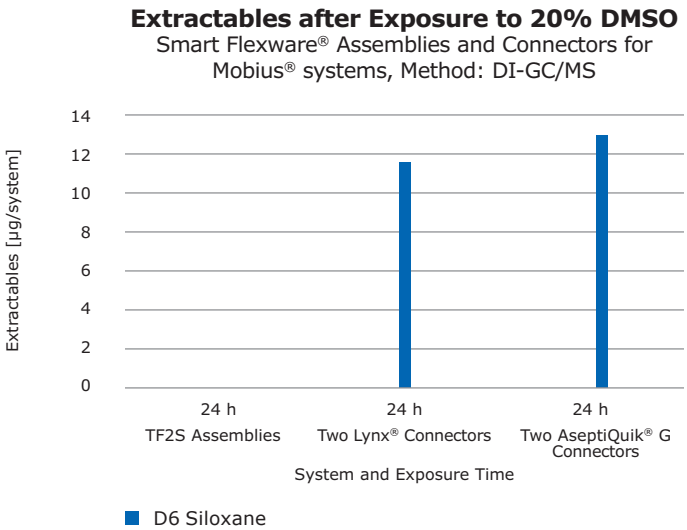


Figure 12: Extractables for Smart Flexware® assemblies and connectors for Mobius® systems, exposed to 20% DMSO

Mobius® FlexReady solution with Smart Flexware® Assemblies for TFF and connectors exposed to 20% DMAc

Figure 13 and Figure 14 show that more individual compounds are detected in samples collected from Mobius® Smart Flexware® Assemblies system and connector flow loop systems after exposure to 20% DMAc as compared to the other two solvents. However, the concentration of each analyte was still below 0.05 µg/mL.

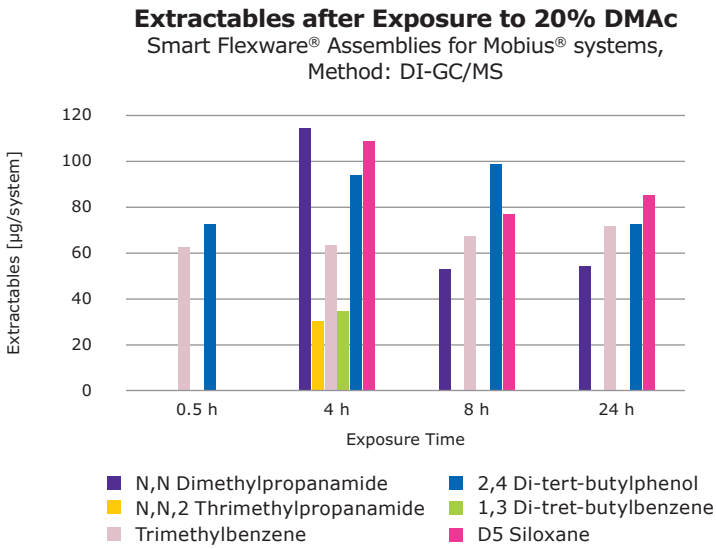


Figure 13: Extractables for Smart Flexware® Assemblies for Mobius® systems exposed to 20% DMAc

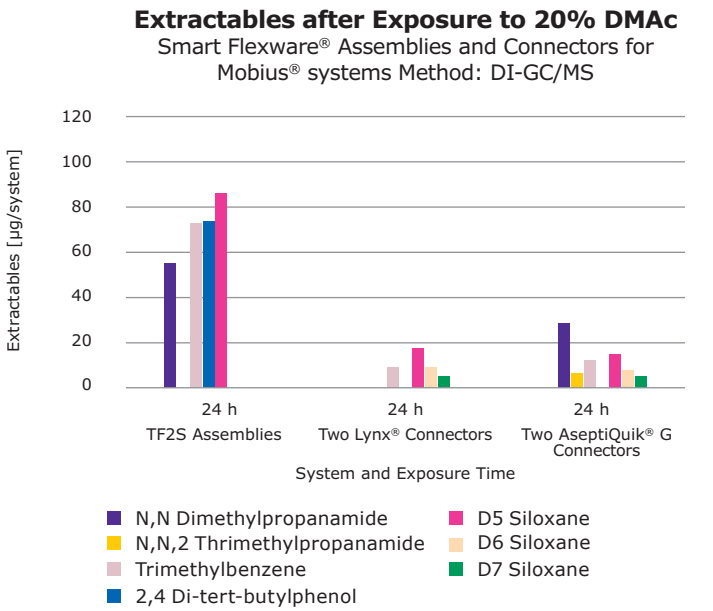


Figure 14: Extractables for Smart Flexware® assemblies and connectors for Mobius® systems, exposed to 20% DMAc

Conclusions

Understanding solvent compatibility in ADC processing is important for the implementation of single use systems. This report indicates that the Mobius® mixer, Mobius® FlexReady Solution with Smart Flexware® Assemblies for tangential-flow filtration or chromatography, Lynx® CDR Connectors, Lynx® S2S Connectors, and Opticap® XL with Millipore Express® SHC membrane are compatible with concentrations of DMSO or DMAc that exceed typical conjugation conditions. In addition, these solvent conditions did not affect the bacterial retention capability of the Opticap® sterile filter or the operational robustness of the materials through extended functional testing as no leaks or physical changes were observed even at high flows and pressures.

Extractables Profile

Mobius® Smart Flexware® Assemblies and Sterile-to-Sterile Connectors

- No peaks were identified by LC-MS or Head Space GC-MS for any of the solvent extractions.
- No metals were identified that were of a risk concern
- Peaks were identified by direct inject GC-MS, although all were below 0.05 µg/mL concentration.

Opticap® XL Capsule with Millipore Express® SHC membrane

- A small number of compounds were identified by DI-GC/MS (4 compounds), HS-GC/MS (1 compound), and HPLC-DAD/MS (2 compounds which were also among the 4 found by DI-GC/MS) for the solvent extractions.
- The results in this study demonstrate that, even with no pre-use flushing, the levels of organic compounds would fall below 0.1 µg/mL in a filtered pool even at a relatively low filter throughput of 150 L/m^2.

References:

[1] Removal of Leachables by Ultrafiltration and Diafiltration, MilliporeSigma, Document Number TB4663EN00

[2] American Society for Testing and Material. Standard Test Method for Determining Bacterial Retention of Membrane Filters Utilized for Liquid Filtration. ASTM Standards on Materials and Environmental Microbiology. 2015; Designation F838-15ae1.

Supporting Documents:

Characterizing Extractables from Mobius® Single Use Assemblies, MilliporeSigma, Document Number PF1141EN00

PureFlex™ and PureFlex™ Plus, Disposable process container films extractables evaluation, MilliporeSigma, Document Number AN1121EN00

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