Application Note

Deviron® C16 and Deviron® 13-S9 Detergents for Solvent/Detergent Viral Inactivation of Plasma in Mobius® Single-Use Containers

Background

Viral safety is crucial for biotherapeutic manufacturing.[1] Human plasma-derived products may be at risk of containing viruses, such as hepatitis C, hepatitis B, and human immunodeficiency virus (HIV), despite extensive screening of donation material.[2,3] For biomanufacturing processes, manufacturers need to implement and demonstrate efficacy of virus clearance operations. Detergent-mediated viral inactivation is widely used for plasma processes due to its efficacy, ease of application, robustness, and low impact on product quality attributes.[4] Plasma processes generally combine detergent, traditionally Triton™ X-100 detergent (4-tert-octylphenolethoxylate), with a solvent, tri-n-butyl phosphate (TnBP), to inactivate enveloped viruses.[4]

Single-use systems are increasingly adopted in biomanufacturing based on increased flexibility, reduced footprint, and capital infrastructure costs. They are also a sustainable alternative through reduced water and chemical usage associated with hardware cleaning. Previous work has demonstrated compatibility of Mobius® single-use containers for solvent/detergent treatment with Triton™ X-100 detergent for virus inactivation in plasma.[5]

New sustainable replacements for Triton™ X-100 detergent have been developed to address REACH compliance, namely Deviron® C16 and Deviron® 13-S9 detergents, which have been shown to be effective for virus inactivation in plasma. This work demonstrates the compatibility of these detergents with virus inactivation in a plasma matrix performed in Mobius® single-use containers.

Specifically, we evaluated

- Chemical compatibility of Mobius® single-use containers with Deviron® C16 and 13-S9 detergents
- Efficacy of virus inactivation using model enveloped viruses (e.g., XMuLV and BVDV)
- Mixing performance

Compatibility of Mobius® Single-Use Containers and Deviron® Detergents

Changes in mechanical properties and chemical compatibility of Mobius® single-use containers with Deviron® detergents were assessed using gamma-irradiated 1 L PureFlex™ film bags, representative of Mobius® single-use containers.

The PureFlex™ film bags were exposed to 1 % detergent (Deviron® C16, Deviron® 13-S9, or Triton™ X 100 detergent) with and without 0.3 % TnBP and incubated for 24 h at 40 °C on an orbital shaker at 50 rpm. Triton™ X-100 detergent was included as a positive control in this study. All detergent solutions were prepared in phosphate-buffered saline (PBS). A protein matrix was not included in the samples to improve detection of extractables, based on the assumption that the presence of protein does not alter the quantity and type of extractables. The extracted solutions were collected into glass vials to test for extractable compounds, and detergent analysis was performed to measure non-specific binding to the bag material. The extracted bags were used for mechanical testing to evaluate the strength properties of PureFlex™ film following exposure to the various chemicals, compared to those of the untreated control.

Extractable Analysis

The extractable analysis was performed using two gas chromatography mass spectroscopy (GC-MS) techniques. volatile organic compounds (VOCs) were detected using a headspace method, whereas semi-volatile organic compounds (sVOCs) were detected by a direct inject method using a solvent liquid-liquid extraction. The total extractables amounts included any individual species detected above the reporting limit of 0.02 µg/cm^2. In the extractables profiling, n-hexane, 1,3-di-tert-butylbenzene, and 2,4-di-tert-butylphenol were detected in all samples (**Table 1**). These are known extractable compounds of gamma-irradiated PureFlex™ film. The total amounts of extractables did not differ between samples obtained from bags exposed to Triton™ X-100, Deviron® C16 and Deviron® 13-S9 detergents with or without TnBP. Moreover, the identified extractables have no known genotoxic properties which suggests that the patient risk of exposure to these compounds is low. In addition, these low molecular weight extractables as well as the solvent/detergent (S/D) chemicals will likely be removed by significant levels in subsequent downstream processing steps, such as chromatography or ultrafiltration/diafiltration, further reducing the potential patient exposure risk.

Table 1. Extractables of PureFlex™ film after exposure to S/D chemicals.

Compound	CAS No.	PureFlex™ Film Extracts (µg/cm²)					
		Without 0.3 % TnBP			With 0.3 % TnBP		
		1% Triton™ X-100	1% Deviron® C16	1% Deviron® 13-S9	1% Triton™ X-100	1% Deviron® C16	1% Deviron® 13-S9
n-Hexane	**110-54-3**	0.13	0.31	0.16	0.40	0.22	0.55
1,3-di-tert-butylbenzene	**1014-60-4**	0.13	0.22	0.12	0.14	0.22	0.24
2,4-di-tert-butylphenol	**96-76-4**	0.25	0.30	0.14	0.10	0.18	0.08
Reporting limit = 0.02 µg/cm²							

Non-Specific Binding of Solvent/Detergent Chemicals to PureFlex™ Films

The non-specific binding or adsorption of detergent to the PureFlex™ film surface was evaluated indirectly by determining the detergent concentration in the solution after incubation in the study described above. The detergent concentrations were quantified using ultra-performance liquid chromatography coupled with mass spectrometry (UPLC-MS). The detergent recovery values ranged from 93 to 106 % as shown in **Table 2**. These results indicated that the non-specific binding to the film surface was very low, therefore all the detergent was available for the viral inactivation process.

Table 2. Amount of detergent recovered after incubation of S/D chemicals in PureFlex™ film bags.

Detergent/Solvent Solutions	Recovery (%; n = 2)
1 % Triton™ X-100 in PBS	101.0 ± 0.7
1 % Deviron® C16 in PBS	97 ± 0
1 % Deviron® 13-S9 in PBS	93 ± 2
1 % Triton™ X-100 + 0.3 % TnBP in PBS	105.0 ± 0.9
1 % Deviron® C16 + 0.3 % TnBP in PBS	105 ± 2
1 % Deviron® 13-S9 + 0.3 % TnBP in PBS	102.0 ± 0.2

Mechanical Properties of PureFlex™ Films

The mechanical strength of the gamma-irradiated PureFlex™ film was evaluated after exposure to S/D chemicals. Tensile testing was performed using a material testing machine zwickiLine Z2.5 (Zwick Roell, Ulm, Germany), equipped with both flat grips and a 100 N load cell. Stress and strain measurements were performed on rectangular film sections (0.5 in x 4.5 in) with an initial grip separation of 1.5 inches and a crosshead speed of 10 in/min. Four specimen samples were analyzed from each bag and the results are provided as the average. Films were analyzed in terms of their elastic (E) modulus, breaking stress and strain at breaking. The results are summarized in **Table 3**. There were no detectable differences between the control (i.e., unexposed film and PBS-exposed film) and the detergent-exposed film. Chemical exposure did not compromise the properties of the PureFlex™ film, indicating that Mobius® single-use containers are compatible for use in the S/D viral inactivation application with Deviron® C16 and Deviron® 13-S9 detergents.

Table 3. Tensile test results for PureFlex™ film.

Detergent/Solvent Solution (n = 2)	E-Modulus (MPa)	Breaking Stress (MPa)	Strain at Breaking (%)
Unexposed Control (NG)	260 ± 30	14.0 ± 0.7	770 ± 20
PBS-exposed Control (G)	250 ± 30	15.0 ± 0.4	845 ± 6
1 % Triton™ X-100 (G)	260 ± 10	14.0 ± 0.6	770 ± 20
1 % Deviron® C16 (G)	260 ± 3	14.0 ± 0.8	770 ± 50
1 % Deviron® 13-S9 (G)	260 ± 20	15.0 ± 0.9	788 ± 6
1 % Triton™ X-100 + 0.3 % TnBP (G)	268 ± 1	15.0 ± 0.3	800 ± 30
1 % Deviron® C16 + 0.3 % TnBP (G)	270 ± 20	15.0 ± 0.4	810 ± 10
1 % Deviron® 13-S9 + 0.3 % TnBP (G)	244 ± 9	14.0 ± 1.0	780 ± 30

NG: Non-gamma-irradiated; G: Gamma-irradiated

Efficacy of Solvent/Detergent Virus Inactivation in Mobius® Single-Use Containers

The effectiveness of viral inactivation was evaluated using tailored PureFlex™ film bags, which are smaller versions of the standard PureFlex™ film bags. This design allows us to simulate the Mobius® single-use containers made with PureFlex™ film on a small scale, thereby reducing the volumes of reagents required for the experiments while minimizing material waste. These tailored bags maintain the same characteristics as the standard PureFlex™ film bags.

Model viruses were used for testing clearance operations in plasma matrices. These include xenotropic murine leukemia virus (XMuLV), representing human immunodeficiency virus (HIV), and bovine viral diarrhea virus (BVDV), which models hepatitis C.

The tailored 30 mL PureFlex™ film bags containing two ports were gamma sterilized (**Figure 1**). Plasma matrix was generated using a standard cryo-precipitation step.[2] Duplicate bags were filled with 20 mL of cryo-poor plasma matrix and spiked with either XMuLV or BVDV. A concentrated pre-mix of TnBP solvent and detergent was added to final concentration of 0.3 % TnBP (v/v) and 1 % of either Deviron® 13-S9 or C16 detergent. A control feed/hold test article without solvent/detergent was included to quantitate virus inactivation as log removal. All test articles were placed on a rocking platform at 31 °C. Samples were collected at 30, 120 and 240 min. A detergent inactivation control was performed in polypropylene tubes, routinely used for viral inactivation characterization. Virus titers were determined using the tissue culture infectious dose 50 % ($TCID_{50}$) assay on appropriate indicator cell lines for each virus. The log reduction value (LRV) was calculated by measuring the difference between virus titers of the feed/hold and the detergent-treated samples at each time point.

Figure 1: Image illustrating the test article PureFlex™ film bag with two ports.

The efficacy of detergent-mediated virus inactivation in PureFlex™ film bags was comparable to polypropylene tubes for both model viruses, i.e. XMuLV (**Figure 2**) and BVDV (**Figure 3**). The Deviron® detergents demonstrated effective inactivation performance with XMuLV showing an LRV > 4.8, while BVDV reached viral titers below the detection limit within 30 minutes, resulting in an LRV > 4.0. The detergent-mediated viral inactivation was consistent across both replicates for each virus.

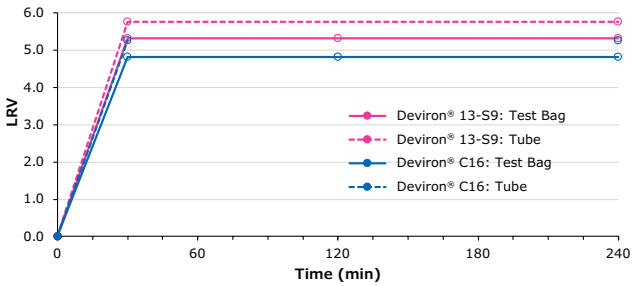

Figure 2: XMuLV inactivation in a plasma matrix (cryo-poor plasma at 31 °C, using 1 % detergent). Solid markers have detectable virus and unfilled indicate virus titers below limit of detection.

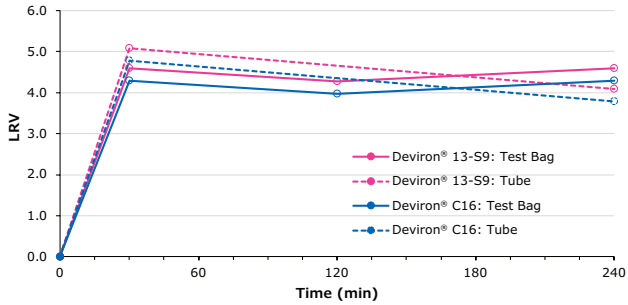

Figure 3: BVDV inactivation in a plasma matrix (cryo-poor plasma at 31 °C, using 1 % detergent). Filled markers have detectable virus, while unfilled indicate virus titers below limit of detection.

Characterization of Solvent/Detergent Mixing in Mobius® Single-Use Containers

Mixing studies were conducted at the 50 L scale in a 5 % (w/v) bovine serum albumin (BSA) in 1x PBS solution using a Mobius® MIX single-use mixing system. Extent of mixing was evaluated on a target concentration of 1 % detergent with and without 0.3 % TnBP. Sampling began with the start of a 10x solvent/detergent concentrate addition through the bottom port at 1 L/min. Efficiency of mixing was determined after sampling for 120 minutes. Samples from the top and middle of the vessel were taken at different intervals at room temperature. The concentration of detergent was determined by liquid chromatography-mass spectrometry (LC-MS). The top and middle sampling showed similar profiles. **Figure 4** shows the results generated from four independent mixing studies at 50 L scale from the middle of the vessel. At the 50 L scale, mixing was complete within 10 minutes or less after the start of the S/D addition. These mixing times were consistent with data from previous detergent studies in Mobius® single-use containers.

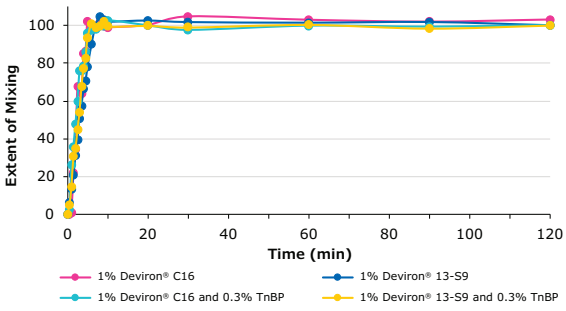

Figure 4: Extent of mixing of S/D chemicals in 50 L Mobius® MIX single-use mixing system in 5 % BSA at 200 rpm.

Summary

This application note demonstrates the suitability of Mobius® single-use containers for solvent-detergent virus inactivation in a plasma matrix with both Deviron® C16 and 13-S9 detergents, offering a flexible solution for a biomanufacturing process.

In summary:

- The Mobius® containers equipped with PureFlex™ films are compatible with both Deviron® detergents, in combination or in absence of the solvent TnBP.
- Model enveloped viruses, such as XMuLV and BVDV, are effectively inactivated in the Mobius® single-use containers.
- Deviron® detergents do not cause additional film extractables, beyond known species generated in aqueous solutions; and levels are low and likely to be removed in the downstream process.
- At 50 L scale, mixing was completed and the desired target concentration of detergent was reached within 10 minutes in a model solution.

The combination of Mobius® single-use containers together with Deviron® detergents offers opportunities in biotherapeutic manufacturing for more environmentally sustainable processes, reduced process contamination risk and increased efficiency with reduced cleaning validation requirements compared to conventional stainless-steel systems.

References

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