

Single-Use Mixing Technology for Virus Clearance (Part 2: Compatibility, Leachables, and Scale-Up Studies)

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ABSTRACT

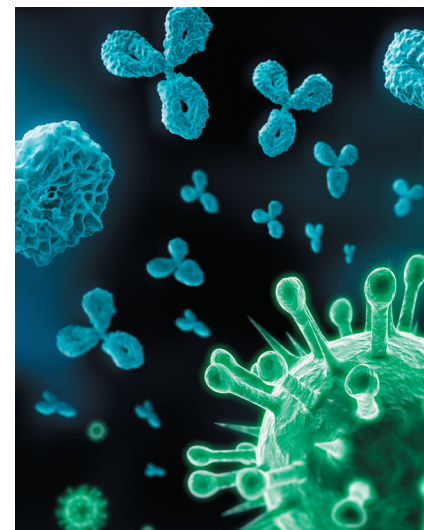
The potency of recombinant proteins such as monoclonal antibodies remains typically unaffected by detergent-based virus inactivation treatments because detergents tend to target lipids and lipid derivatives rather than proteins. Virus inactivation treatments that use detergents are robust with respect to temperature, which allows these steps to be performed at room temperature without thermoregulation. In this two-part article, the authors describe the testing performed to demonstrate the applicability of single-use mixing technology for a virus inactivation step performed using detergent.

As the authors described in Part 1 (1) of this paper, solvent or detergent treatments for virus inactivation were originally developed for use in the manufacture of blood products (2, 3). They do not require expensive reagents or expensive equipment. The method inactivates enveloped viruses by solubilizing the viral envelope's lipid membrane structure, which prevents the virus from binding to or infecting cells. The potency of recombinant proteins such as monoclonal antibodies (mAbs) is typically unaffected because the detergent targets lipids and lipid derivatives rather than proteins (4). Virus inactivation treatments that use detergents such as Triton X-100 (MilliporeSigma) are robust with respect to temperature, which allows these steps to be performed at room temperature without thermoregulation. The solvent that has been added must be removed after the step, often by chromatography, to prevent it entering the final product.

Biomanufacturers must be confident that virus inactivation steps using deter-

gents are compatible with single-use technologies that may be used for this step. In this two-part article, the authors describe the testing performed to demonstrate the applicability of single-use mixing technology for a virus inactivation step performed using detergent (Triton X-100, MilliporeSigma).

The study was performed in a series of steps. Part one of this paper (1) described the study of the inactivation of enveloped viruses (xenotropic murine leukemia virus [X-MuLV], bovine viral diarrhea virus [BVDV], and pseudorabies virus [PRV]) by detergent treatment (Triton X-100, MilliporeSigma) within a single-use bag (Flexel, Sartorius Stedim Biotech). Testing showed that no significant adsorption of the detergent to the bioprocess container occurred. Enveloped virus spiking studies showed that a minimum reduction factor of $3.84 \log_{10}$ could be expected in this single-use step and that, in most cases, the reduction factor would be greater than $\geq 5.01 \log_{10}$.



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Table I. Rationale for the parameters selected during the studies.

Exp Experiment	Process conditions	Adsorption	Virus clearance	Chemical compatibility	Leachables	Adsorption and mixing efficiency
Bag size	200 L	75 mL	75 mL	500 mL	500 mL	50 L
Detergent (Triton X-100, MilliporeSigma) concentration (w/v %)	0.9 to 1.2	1	0.7	20	1	1
	Process condition	Process condition	Worst case	Storage condition	Process condition	Process condition
Surface-volume ratio (cm ² /mL)	0.2	3.5	3.5	1.2	1.2	0.2
	Process condition	Worst case	Worst case	Worst case	Worst case	Process condition
Contact time (hours)	1	2	1	72	24	2
	Process condition	Worst case	Process condition	Worst case	Worst case	Worst case
Temperature (°C)	20 ± 5	20 ± 5	14±1	5±3	30±5	20 ± 5
	Process condition	Process condition	Lower limit	Storage condition	Upper limit	Process condition
Continuous mixing	Yes	Yes	Yes	No	No	Yes

In Part 2 of this paper, the authors will describe chemical compatibility and leachable studies performed on the single-use bags (Flexel, Sartorius Stedim Biotech) for the magnetic mixer in the presence of detergent (Triton X-100, MilliporeSigma). Finally, the authors will describe the evaluation of film adsorption and detergent homogeneity in a 50-L magnetic mixer to verify that the results generated at the small scale can be translated to an industrial scale operation.

STUDY DESIGN

At the production-scale, the virus inactivation of a neutralized Protein A mAb-eluate is performed using detergent (Triton X-100, MilliporeSigma). The inactivation is performed at a final concentration of 1% (w/v) detergent for one hour at room temperature (20 ± 5 °C) in a magnetic mixer (Sartorius Stedim Biotech). A typical processing volume of 85 L of mAb is treated in a 200-L single-use bag (Flexel, Sartorius Stedim Biotech), constructed from S40 film (Sartorius Stedim Biotech). The S40 film is a multi-layer film which includes an ethylene vinyl alcohol (EVOH) gas barrier and a polyethylene (PE) fluid contact layer.

The critical parameters for the inactivation step are the detergent concentration, product-detergent contact time, the film surface area-to-product volume ratio, and the temperature. The adsorption, chemical compatibilities, and leachable studies were performed under worst-case contact time conditions (see **Table I**).

Experiments were performed by LFB Biomanufacturing in collaboration with Sartorius Stedim Biotech Confidence Validation Services.

DESCRIPTION OF THE SCALE-DOWN MODELS

A detailed description of scale-down models is provided in Part 1 of this paper (1). Briefly, the scale-down models used in the studies were representative of the single-use bags (Flexel, Sartorius Stedim Biotech) for the magnetic mixer (**Table II**). An equivalent welding process was used to attach tubing to the scale-down bag chamber as is used for the single-use bags. Additional impeller parts, manufactured from representative materials of construction, were added inside the scale-down bag to mimic the contact between the processing solution and the magnetic mixer impeller.

METHODS

Chemical compatibility

The mechanical resistance of the single-use bags (Flexel, Sartorius Stedim Biotech) to be used with the magnetic mixer was evaluated following exposure to 20% detergent (w/v) (Triton X-100, MilliporeSigma) for 72 hours at 5 °C ± 3 °C.

The test mimics the concentrated detergent (Triton X-100, MilliporeSigma) solution storage conditions that would be applied during a manufacturing campaign. At process scale, the solution is stored in concentrated form and then subsequently diluted to provide the 1% (w/v) detergent solution used for virus inactivation. The inactivation step is performed with 1% detergent at 20 ± 5 °C for one hour. Although processing occurred at a higher temperature than the storage mimic, the storage time in process conditions was 72-fold shorter and the concentration of the detergent was 20-fold less. According to the ASTM F1980 *Standard Guide for Accelerated Aging of Sterile Barrier Systems for Medical Devices* (5), 72 hours at 5 °C correspond to 25.7 hours at 20 °C. Thus, it was judged that the storage conditions were worst-case conditions for the chemical compatibility study.

Table II. Characteristics of commercial and scaled-down single-use bags (Flexel, Sartorius Stedim Biotech). Detergent used was Triton X-100 (MilliporeSigma).

Material description	200-L bag for magnetic mixer	50-L bag for magnetic mixer	500-mL scale-down bag	75-mL scale-down bag
Study	In process	Detergent-homogeneity during mixing and adsorption	Chemical compatibility and leachables	Detergent film adsorption, virus inactivation studies
Film material	S40 film	S40 film	S40 film	S40 film
Configuration	3D	3D	2D	2D
Sterilization	Irradiation at 25-45 kGy	Irradiation at 25-45 kGy	Irradiation at 25-45 kGy	Irradiation at 25-45 kGy
Agitation method	Magnetic mixer	Magnetic mixer	Static	Orbital shaker
Surface-volume ratio	0.2 cm ² /mL	0.2 cm ² /mL	1.2 cm ² /mL	3.5 cm ² /mL
Impeller material	Yes	Yes	Fragment	Fragment

The test was performed by adapting the standard test method for resistance of plastic to chemical reagents (ASTM D543) (6). The test articles

were a 500-mL single-use bag (Flexel, Sartorius Stedim Biotech) with PE port, silicone tubing, and PE impeller parts. The contact surface-to-vol-

ume ratio of the scale-down mimic was 1.2 cm²/mL compared to the 0.2 cm²/mL ratio for the 200-L bag that would be used at the large scale. The scale-down experiment was, therefore, considered to represent worst-case processing conditions. The 500-mL single-use bag was gamma-irradiated prior to the experiment at 25–45 kGy, which was also representative of the large scale. A control was prepared by replicating the small-scale experiment but replacing the 20% detergent (Triton X-100, MilliporeSigma) solution with purified water.

To demonstrate the chemical compatibility, a series of tests were performed following incubation with the detergent: a visual inspection allows evaluation of the impact of the solution on the plastic, and criteria such as transparency; film brittleness; cracking; presence of bubbles; and surface damage, such as decomposition and delamination, were also evaluated. Additionally, a weight-loss

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Table III. Chemical comparability testing results of the 500-mL scale-down mimic of the 200-L single-use bags (Flexel, Sartorius Stedim Biotech) with 20% detergent (Triton X-100, MilliporeSigma) solution at 5 °C ±3 °C.

Test category	Test description		Specification	Meets specification?
N/A	Weight-loss analysis on bags and accessories		Weight loss is less than 1%	Pass
Category 1	Visual inspection of bag and impeller: Transparency/opacity		No transparency/opacity modification	Pass
	Visual inspection of bag and impeller: color modification		No color modification	Pass
Category 2	Film thickness		175–225 µm	Pass
	Polyethylene seat dimensions		External diameter: 110.49–111.55 µm Internal diameter: 8.46–8.72 µm	Pass
Category 3	Film tensile strength		Machine direction tensile strength > 40 N Traverse direction tensile strength > 40 N	Pass
	Tensile strength of the film seals		Tensile strength > 80 N	Pass
	Tensile strength of the film/port seals		Tensile strength > 80 N	Pass
	Tensile strength of the port/tubing connections		Tensile strength > 80 N	Pass
	Tensile strength of the tubing/connector connections		Tensile strength > 80 N	Pass
Category 4	Visual inspection of the bag and impeller: cracking/bubbles		No cracking/no bubble	Pass
	Visual inspection of the bag: delamination		No delamination	Pass
Category 5	Drop test	Checking for leaks on component connections	No leaks on component connections	Pass
		Checking for leaks on the bag seals	No leaks on bag seals	Pass
		Checking for leaks on the film/port seals	No leaks on film/port seals	Pass
		Checking for leaks on the port/tubing connections	No leaks on the port/tubing connections	Pass
	Visual inspection of the bag and impeller: decomposition		No decomposition by dissolution	Pass
	Visual inspection of the bag: brittleness		No brittleness	Pass
	Visual inspection of the bag and impeller: crater/hole		No crater/hole	Pass

analysis was performed to measure any potential reduction of bag barrier properties. Mechanical properties were tested by tensile-strength testing the film material on the sealing parts and on the connections. The results were then compared to specifications. Stress-test conditions were applied using a drop test to evaluate the mechanical resistance of the container after the interaction with the 20% (w/v) detergent solution.

Interactions between bag components and the detergent were also evaluated by verifying that the dimen-

sions of major components were still within the required specifications following contact with the test solution. Finally, spectral properties of the film were verified and compared to the control sample in contact with water.

Leachable study

The leachables from the single-use bags (Flexel, Sartorius Stedim Biotech) for the magnetic mixer constructed from S40 film were evaluated by performing an extraction with a 1% (w/v) detergent (Triton X-100, MilliporeSigma)

solution. Biomanufacturers can also be concerned about particulates generated by mixing operations, which can ultimately lead to aggregate formation. The proportion of monoclonal antibody monomer in the final product of this process, however, exceeded 99%, and an analysis of particulates was outside the scope of this study.

The 500-mL scale-down, irradiated bag with the same design as that used in the chemical compatibility study was used. A 24-hour incubation of the solution was performed

Table IV. Results from the extraction with 1% detergent (Triton X-100, MilliporeSigma) solution on a single-use bag (Flexel, Sartorius Stedim Biotech) with impeller parts.

Analysis	Method	Result
Element impurities	ICP-MS/OES	Potassium and zinc detected at quantifiable and equivalent levels in the blank and bag extract
Organic leachables	RP-UHPLC-UV	No leachables detected
Organic leachables	GC-MS	Two hydrocarbon leachables detected at an estimated concentration of 1 ppm each
ICP-MS/OES is inductively coupled plasma mass spectrometry/optical emission spectrometer. RP-UHPLC-UV is reversed phase ultra-high-performance liquid chromatography with ultraviolet detection. GC-MS is gas chromatography-mass spectrometry.		

at 30 ± 5 °C, under static conditions, thereby ensuring that the upper end of the production-scale temperature specification of 20 ± 5 °C was covered. A control was performed by replicating the small-scale experiment but replacing the 500-mL bag with a glass bottle and a polytetrafluoroethylene (PTFE) bottle incubated under the same conditions.

Process solution and control samples were analyzed by inductively coupled plasma-mass spectrometry (ICP-MS)/optical emission spectrometer (OES), reversed phase ultra-high-performance liquid chromatography with ultraviolet detection (RP-UHPLC-UV), and gas chromatography-mass spectrometry (GC-MS). Element impurities analysis was performed by ICP-MS to evaluate the migration of inorganic compounds from the plastic materials (35 elements were evaluated according to the ICH Q3D, EMA, and USP <232> requirements) (7, 8, 9). The GC-MS analysis was used to detect volatile and semi-volatile organic leachables, and the UHPLC-UV provides additional information by screening the UV-active organic compounds independently of their molecular weight. The combination of these three analytical methods provides a comprehensive leachable profile.

Translation to industrial scale

A study was performed to verify that the detergent (Triton X-100,

MilliporeSigma) concentration was homogeneous throughout a 50-L single-use 3D bag (Flexel, Sartorius Stedim Biotech) once agitated by the magnetic mixer. Both adsorption and effective mixing were verified under process conditions at a scale that was 25% of the final production scale volume. The findings are not generalizable for different agitation rates or mixer working volumes. The study was performed with purified water instead of Protein A eluate, which was considered legitimate under the assumption that differences in the respective viscosities of water and the protein solution would be negligible. The protein solution had a concentration of less than 10 g/L.

A 50-L single-use bag was filled with 50 L of purified water representing a 'worst-case scenario' for the film surface area in contact with product. Detergent (Triton X-100, MilliporeSigma) was added to the single-use bag (Flexel, Sartorius Stedim Biotech) to a final concentration of 1% (w/v) via the top port of the bag using a peristaltic pump and a calibrated scale to ensure that the required quantity of detergent was added. For GMP operations under aseptic conditions, detergent addition can be performed using a dedicated sample port at the bottom of the bag. The mixing rate was 40 rpm. This gave efficient mixing but did not create vortices or foam. Individual sam-

ples were taken after 0, 30, 60, and 120 minutes at a point that was 30 mm below the liquid surface and from a sample port at the bottom of the bag. In this way, the detergent concentration could be assessed at the top and bottom of the container. Once mixing was established and the solution appeared homogeneous, an initial sample was taken at time = 0 minutes.

A control experiment was performed in parallel in a 5-L borosilicate glass bottle with 2105 g of 1% (w/v) detergent (Triton X-100, MilliporeSigma) in purified water. A magnetic mixing plate and stirrer bar was used to mix the solution. The protocol was the same in all other respects. Samples were taken after 0 and 120 minutes.

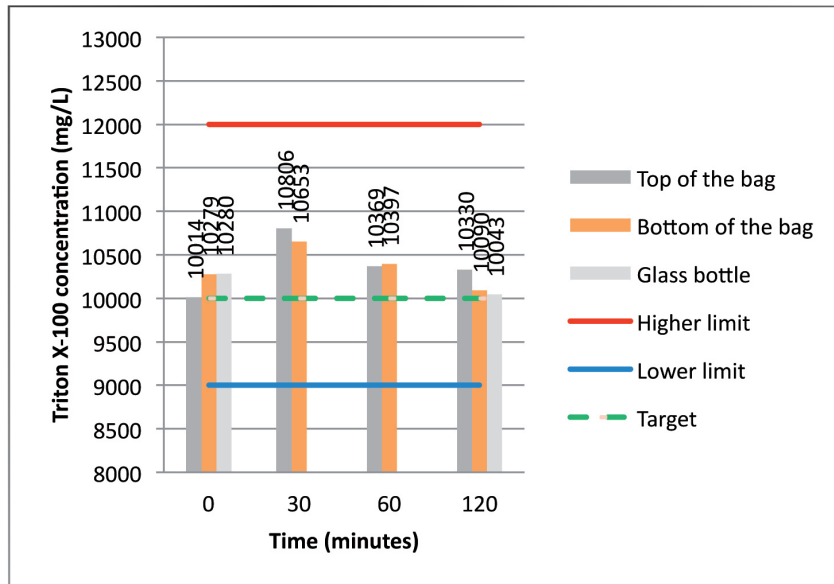
Sampling was performed using 30-mL sterile glass vials, which were immediately stored at 2–8 °C. The detergent concentration was measured by RP-UHPLC.

RESULTS

Chemical compatibility

The results of a chemical compatibility study between the single-use bags (Flexel, Sartorius Stedim Biotech) used in conjunction with the magnetic mixer and a 20% (w/v) detergent (Triton X-100, MilliporeSigma) solution are shown in **Table III**. A category number indicating the criticality of the test is attributed to each test. Higher category numbers indicate a higher level of criticality. The values for all the tests are combined within an overall chemical compatibility calculation score. This scoring system is based on previous experience and product-use knowledge. The results from the tests show that the scale-down mimics comprising single-use bags and impeller parts demonstrate excellent chemical compatibility when exposed to 20% detergent solution for 72 hours at 5 °C ± 3 °C. This provides further confidence that the single-use bags for the magnetic mixer will provide a suitable container for the large-scale virus inactivation step.

Figure 1. Detergent (Triton X-100, MilliporeSigma) concentration in the 50-L single-use bag (Flexel, Sartorius Stedim Biotech) and glass bottle over time.



Leachable study

Table IV shows the results of the leachables study between the single-use bag (Flexel, Sartorius Stedim Biotech) and 1% detergent (Triton X-100, MilliporeSigma) solution. Although potassium and zinc were detected in the extract solution, they were also detected at the same level in the blank control sample and are considered matrix artifacts. No leachables were detected by the UHPLC–UV method of analysis. Under scale-down conditions, two organic leachables were detected by GC–MS, each with an estimated concentration of 1 ppm which, when combined, give a total leachable concentration of 2 ppm.

These results were extrapolated to the manufacturing process conditions by multiplying the scale-down leachable concentration by the film surface area/volume ratio expected at process scale and dividing by the film surface area/volume ratio encountered during scale-down. This extrapolation gives an anticipated total leachable concentration at the production-scale of approximately 0.3 ppm. The hydrocarbons detected by GC–MS at retention times of 14.2 and 17.1 minutes are most probably

intermediate molecular weight C13 and C14 compounds. These would most likely pass through the permeate of subsequent ultrafiltration/diafiltration steps or be removed in the flow-through of subsequent bind and elute chromatography steps.

Translation to industrial scale

Figure 1 shows the results from the detergent (Triton X-100, MilliporeSigma) homogeneity study. All data points were close to the target specification of 1% detergent solution (w/v) and within the specified limits of 9000–12,000 mg detergent.

The difference in detergent concentration between the upper and lower sampling locations within the 50-L bag was 2.7% and therefore did not exceed a maximum specified limit of a 10% difference in concentration. The ability of the magnetic mixer to ensure a homogeneous environment within the bag, therefore, was demonstrated. The concentration of detergent was deemed stable over time in the single-use bag, as the greatest difference between lower and higher measured concentrations was 7.90%, which

was within the specified limits of 0 to 10%. The average detergent concentration obtained in the 50-L bag did not exceed a 2.86% difference between that observed in the glass bottle control, thus it is compliant with the $\leq 10\%$ difference limit.

This study demonstrated that the distribution of detergent (Triton X-100, MilliporeSigma) in the mixing bag is homogeneous even under the worst-case conditions for adsorption of high film contact surface area/volume ratio. To consolidate the results and optimize the set-point for mixing rate, the detergent concentration was measured at 0 minutes and 60 minutes, at the lower and upper part of the bag, during the production of the first mAb batch performed at industrial scale.

CONCLUSION

In this study, the use of a single-use mixing technology was evaluated and characterized for the performance of a detergent-based virus inactivation step during a mAb production process. Testing showed that no significant adsorption of detergent (Triton X-100, MilliporeSigma) to the bioprocess container occurred. Enveloped virus-spiking studies demonstrated that a minimum reduction factor of $3.8 \log_{10}$ could be expected in this single-use step and that, in most cases, the reduction factor would be greater than $\geq 5.0 \log_{10}$. Further tests showed that the single-use bags (Flexel, Sartorius Stedim Biotech) for the magnetic mixer had excellent chemical compatibility with a 20% solution of the detergent and that leachables were negligible when extractions were performed with 1% detergent solution. Finally, a scale-up study showed that the magnetic mixer provided sufficient agitation to ensure detergent homogeneity within the single-use bag.

The data obtained during this study was considered compelling, and LFB

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SINGLE-STEP INFLUENZA AND DENGUE VIRUS PURIFICATION

CHT XT is a new mixed-mode chromatography media. It has been designed for superior physical robustness to ensure it can be used repeatedly over a large number of cycles. It maintains the unique separation properties of CHT for aggregate removal and mAb purification. Here we performed single-step purifications of influenza and dengue viruses using CHT XT Media. **Bio-Rad Laboratories, Inc.** www.bio-rad.com/chtxt

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Biomanufacturing plans to implement the virus inactivation step using single-use technology as part of their mAb production platform.

Note: A recent amendment of Annex XIV in the European Union (EU) REACH regulation (10) will lead to the substance 4-(1,1,3,3-tetramethylbutyl)phenol, ethoxylated being prohibited from Jan. 4, 2021 unless an authorization is granted. The Triton X-100 (MilliporeSigma) family is included in this new amendment based on the fact that it degrades to the substance mentioned above. The amendment is due to concerns over the environmental impact of the compound's degradation products. For this reason, European Union biomanufacturers must seek alternatives to Triton X-100 with equivalent efficacy and safety. The research in this article is valuable

for biomanufacturers not affected by the EU REACH regulation, and the approach is informative for biopharmaceutical companies inside of the EU considering using other detergents for virus inactivation steps.

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REFERENCES

1. R. McCarthy, et al., *Biopharm International* 31 (10) 26–33 (2018).
2. A.M. Prince, et al., *Eur J Epidemiol.* 3: 103-18 (1987).

3. Y.T. Hsieh, et al., *Transfusion* 56: 1384–1393 (2016).
4. K.M. Remington, "Fundamental Strategies for Viral Clearance Part 2: Technical Approaches," *bioprocessintl.com*, May 12, 2015.
5. ASTM, F1980, *Standard Guide for Accelerated Aging of Sterile Barrier Systems for Medical Devices*, ASTM International, 2007
6. ASTM, D543: *Standard Practices for Evaluating the Resistance of Plastics to Chemical Reagents*, ASTM International, Nov. 1, 2014.
7. ICH, Q3D *Guideline for Elemental Impurities*, *International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use*, Dec. 16, 2014.
8. EMA, *Guideline on the Specification Limits for Residues of Metal Catalysts or Metal Reagents* (London, Sep. 2008).
9. USP General Chapter <232>, "Elemental impurities—Limits, USP 39" (US Pharmacopeial Convention, Rockville, MD, 2016).
10. ECHA, "Authorisation List," <https://echa.europa.eu/authorisation-list/-/dislist/details/0b0236e1807df80d>, Sept. 2018. ♦