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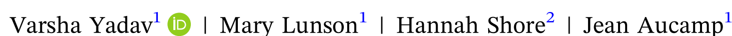
Systematic Development of a Detergent Toolbox as an Alternative to Triton X-100



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ABSTRACT

Detergents are routinely included in protein purification processes to inactivate enveloped viruses that may arise from adventitious or endogenous contamination. The detergent Triton X-100 (TX-100) has been widely used as part of the production process for therapeutic proteins. However, recent ecological studies indicate that TX-100 and its metabolites detrimentally impact aquatic organisms, thus alternative detergents for viral inactivation are required. The overall aim of this study was to identify one or more detergents that are a suitable replacement for TX-100 in the viral inactivation step. In stage one, 16 potential alternatives were identified and screened against TX-100 using multiple criteria such as solubility, feasibility of virus inactivation, critical micelle concentration, and storage conditions. The multi-criteria decision analysis (MCDA) methodology was used to identify four candidates for the second stage assessment. In stage two, a detailed evaluation was undertaken and two candidates C16-AO, and C11/15-sEO9, were found to be practical alternatives to TX-100 for use in protein therapeutic production processes for inactivating enveloped viruses. In addition, C13-EO8 demonstrated good viral inactivation capability and warrants further investigation in detergent clearance and impact on product quality.

1 | Introduction

Mammalian cell culture is pivotal in the biomanufacturing of therapeutic proteins and viral vaccines. Viral contamination is an inherent risk of cell culture production processes (Aranha 2012; Chadd and Chamow 2001; Farshid et al. 2005; Hesse and Wagner 2000). Viruses may be introduced into cell culture-derived products from a contaminated cell bank (endogenous contamination) or during production (adventitious contamination) (Kerr and Nims 2010; Remington 2015). Therefore, it is essential to inactivate and remove viruses from the production process as outlined in ICH Q5A(R2) (ICH 1997).

The clearance of viruses from cell culture-derived protein therapeutics is achieved by either chemical inactivation with low pH, solvents/detergents or physical removal during purification processes (Cameron and Smith 2014; Polasek et al. 2023; Shukla and Aranha 2015; Zhou 2009). Low pH is the preferred viral inactivation (VI) method due to simplicity. However, some proteins have limited stability at low pHs, causing aggregation and partial denaturation, therefore, detergent treatment is an effective alternative (Joshi et al. 2022; Wälchli et al. 2020). TX-100 and the tri-n-butyl phosphate (TNBP)/Polysorbate 80 (Tween 80) solvent/detergent mixture are the predominant detergent treatments used in the cGMP manufacture of biotherapeutics.

TX-100 is a nondenaturing detergent that solubilizes lipid membranes and is widely used in manufacturing because of its desirable chemical properties (e.g., low critical micelle concentration (CMC), low-hydrophilic lipophilic balance (HLB), relatively low viscosity, good compatibility with biopharmaceutical products) and availability in compendial grades from high-quality raw material vendors (Luo et al. 2020). However, due to its detrimental effects on the environment (European Chemical Agency [ECHA] 2017; White et al. 1994), TX-100 has been added to the ECHA "candidate list of substances of very high concern

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Therefore, the industry is keen to replace TX-100 in VI applications and several detergent candidates have shown promise in screening assays (Chen et al. 2020; Conley et al. 2017; Farcet et al. 2019; Luo et al. 2020). Alternatives to TX-100 include pH-neutral arginine buffer (McCue et al. 2014), caprylate (Lundblad and Seng 1991), lauryldimethylamine-oxide (LDAO) (Conley et al. 2017), Ecosurf (Fisher et al. 2016), Nereid (Farcet et al. 2021) PEG 9 Lauryl Ether and PEG 6 Caprylic/Capric Glycerides (Hunter et al. 2021), Simulsol SL 11 W (Luo et al. 2020), amongst others. The replacement detergent should be readily interchangeable with TX-100 for implementation in manufacturing and the conversion of legacy processes. In addition, it should have high solubility, low viscosity and be available in a suitable reagent grade and quantity to meet the demands of large-scale manufacturing.

It has also been observed that some new molecular formats (NMFs) are not compatible with one or more of the virus inactivation options: low pH, non-ionic or ionic detergents. The detergent properties resulting in effective viral inactivation or protein instability are not well understood. Therefore, it is essential to develop a toolbox and identify and characterize more than one alternative substance candidate to TX-100, to support the manufacture of a wide range of NMFs.

In this study, we assessed 16 environmentally friendly detergent candidates and TX-100 as control, in a two-stage approach. The first stage of this study involved the selection of lead candidates from a panel of non-ionic and zwitterionic detergent candidates. The second stage focused on a detailed evaluation of the shortlisted detergents, where important process parameters, such as detergent concentration and treatment time, were studied using suitable viral inactivation assays. Furthermore, we investigated the ability of protein A column chromatography to remove these detergents from the feedstream.

We report on the identification and evaluation of two eco-friendly detergents for inactivating enveloped viruses which can be implemented in large-scale mammalian manufacturing processes.

2 | Materials and Methods

2.1 | Detergents

All detergents were acquired or supplied by Arxada, Merck, or Santa Cruz Biotechnology. The 16 detergents and TX-100 control evaluated in this study are summarized in Table 1 and include non-ionic, and zwitterionic agents. The C11/15-sEO9 listed in Table 1 was evaluated in the technical grade available as TERGITOL 15-S-9 with a CAS no. 84133-50-6. Stock detergent solutions were added to the virus inactivation experiments on a weight-to-weight (w/w) percentage basis.

2.2 | CMC Measurements

CMC values are typically determined in water. However, in manufacturing processes, the detergent will be used with harvested cell culture fluid (HCCF). Therefore, a modified cell culture medium (CCM) without any surfactants was chosen as the solute to determine the CMC values under conditions more representative of the process environment. A microtiter plate-based fluorescence assay, using Nile Red, was developed to measure CMC values. All experiments were performed at ambient temperature.

2.2.1 | Method Development

The fluorescence measurement were made in Corning 3925 96-well solid black microplates, using a SpectraMax M5e plate reader and SoftMax Pro 7 analysis software from Molecular Devices. The concentration of Nile Red was evaluated in the range of 1.25–40 μg/mL. Emission and excitation wavelength scans were performed in the ranges of 440–540 and 580–680 nm, respectively. TX-100 was used as a representative detergent for method development.

2.2.2 | Detergent CMC Determination

A series of concentrations was prepared for each detergent using a 1:2 dilution and a well fill volume of 160 μL. The dilution range was determined using the reported CMC value as reference. A volume of 10 μL Nile Red stock solution was added to the detergent dilution series to give a final dye concentration of 5 μg/mL. Sealed microtiter plates were incubated overnight (16–24 h) at ambient temperature on an orbital plate shaker. Fluorescence measurements were made at the excitation and emission wavelengths of 537 and 636 nm, respectively. Inflection point analysis of the fluorescence signal was used to determine the CMC values.

2.3 | Viruses

The two model enveloped viruses were evaluated in this study, Pseudorabies virus (PRV) from the family of Herpesviridae, and the Xenotropic Murine Leukemia virus (X-MuLV) a retrovirus. Virus stocks were produced from infected susceptible cell lines Vero (ECACC, 84113001) for PRV studies and PG4 (ATCC, CRL-2032) cell lines for X-MuLV studies.

2.4 | Virus Inactivation Experiments

In stage one, the efficacy of virus inactivation was determined following a fixed treatment period (screening study), while in stage two, the rate of virus inactivation was assessed (kinetic study). Both the viral inactivation screening and kinetic studies were conducted using mAb 1 (IgG4) HCCF. The necessary measures and controls were implemented to ensure that the detergents and HCCF were not toxic to indicator cells and did not interfere with viral replication in the assay.

2.4.1 | Screening Studies

The virus inactivation screening studies evaluated the 16 detergents and TX-100 as a control where all detergents were

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TABLE 1 | Details of detergents used in the study.

Detergent chemical name	Abbrevation	Trade name	CAS number	Type	Supplier
Dimethyl didecylphosphine oxide	C12-PO	ND	871-95-4	Non-ionic	Santa Cruz Biotechnology
Octaethylene glycol monododecyl ether	C12-EO8	ND	3055-98-9	Non-ionic	Merck
Iso-tridecyl alcohol polyglycol ether	C13-EO8	Genapol X-080	9043-30-5	Non-ionic	Merck
Octyl-β-D-glucopyranoside	C8-GLU	ND	29836-26-8	Non-ionic	Merck
N,N-Dimethyltetradecylamine *N*-oxide	C16-AO	Deviron C16	3332-27-2	Non-ionic	Merck
C_{11}-C_{15} secondary alcohol ethoxylate	C11/15-sEO9	Tergitol 15-S9 Deviron 13-S9	68131-40-8[a]	Non-ionic	Merck
Polyglyceryl-10 Caprylate/Caprate	C8/C10-GLY10	SYNETH C 15	68937-16-1	Non-ionic	Arxada
Polyglyceryl-10 Laurate	C12-GLY10	SYNETH L 15	74504-64-6	Non-ionic	Arxada
Polyglyceryl-10 Oleate	C18-GLY10	SYNETH O13	9007-48-1	Non-ionic	Arxada
n-Dodecanoylsucrose	C12-SUC	ND	25339-99-5	Non-ionic	Merck
t-Octylphenoxypolyethoxyethanol	TX-100	Triton X-100	9036-19-5	Non-ionic	Merck
3-([3-Cholamidopropyl] dimethylammonio)-2-hydroxy-1-propanesulfonate	CHAPSO	ND	82473-24-3	Zwitterionic	Merck
N,N-Dimethyldecylamine *N*-oxide	C12-AO	ND	2605-79-0	Zwitterionic	Merck
3-(Decyldimethylammonio) propanesulfonate inner salt	C10-SB	ND	15163-36-7	Zwitterionic	Merck
3-Dodecylamido-*N,N′*-dimethylpropyl amine oxide	C12-APAO	ND	61792-31-2	Zwitterionic	Merck
Dodecyldimethylammonio)acetate	C12-AA	ND	683-10-3	Zwitterionic	Merck
N-(Carboxymethyl)-*N,N*-dimethyl-3-[(1-oxododecyl)amino]-1-propanaminium Inner Salt	C12-APB	ND	873943	Zwitterionic	Santa Cruz Biotechnology

[a]Alcohols, C11-15-secondary, ethoxylates is a broad group of substances, for example, homologues with different alkyl chain lengths and number of ethylene oxides. Several trade names can be found under the given CAS No. ND, No data.

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evaluated at a single concentration (Supporting Information S1: Table S.I). PRV was spiked into the HCCF-detergent mixture at a ratio of 5% v/v and incubated for 120 min at $15 \pm 1°C$. Virus titer were quantified using end-point titration (EPT) and large volume plating (LVP). The detergents were tested at concentrations of 5–50-fold the reported CMC value (Supporting Information S1: Table S.I).

2.4.2 | Kinetic Studies

The virus inactivation kinetic study was performed on four selected candidates—C13-EO8, C12-GLY10, C11/15-sEO9, C16-AO and control, TX-100. The stock solutions of the detergents were prepared and added as 1% w/w to the HCCF to achieve a desired detergent concentration (Supporting Information S1: Table S.II). PRV or X-MuLV were spiked into this mixture at a ratio of 10% v/v and incubated for < 1-, 10-, and 60-min time-points at $15 \pm 1°C$. The virus titer quantification was performed using EPT for all time points along with LVP for the 10 and 60 min time points. The kinetic studies were used to determine the minimum incubation time required to achieve the desired $\log_{10}$ reduction.

2.5 | Toxicological and Process Impurity Risk Assessments

For the toxicological assessments, the mechanism of action or class effect and the dose–response with potential systemic toxicity were considered. Whenever systemic toxicity, and hazard or pharmacodynamic effects were identified, a permitted daily exposure intravenous (PDE IV) was calculated. When the overall toxicity was considered as low potential, the default limit from the ICH guideline for residual solvents Q3C(R8) with low toxic potential (Class 3) were applied (ICH 2018). The PDE intravenous (PDE IV) was used to calculate the required safety factors and to evaluate if the detergents can be reduced to below permissible levels. The process-related impurity (PRI) risk assessment approach described by Lou et al. (2021) was used to determine the safety risk and strategy to demonstrate detergent clearance.

2.6 | Detergent Assay

Detergent assay development for C12-GLY10, C16-AO, and C11/15-sEO9 was performed by Eurofins Agroscience Services EAG Laboratories GmbH. A Sciex Triple Quadrupole 3500 LC-MS/MS system, equipped with TurboIonspray ESI source was used along with a Kromasil C18 HPLC column (length: 100 mm, i.d.3 mm, particle size: 5 μm).

2.7 | Detergent Clearance Assessment

The removal of detergent from the feedstream is essential for use in a biopharmaceutical manufacturing process as any carryover into the final drug product could pose safety concerns. Detergent clearance and product quality runs were performed at laboratory scale using three diverse monoclonal antibodies (mAb) (Table 2) to demonstrate broad product application. The process intermediates from the lab scale and pilot scale protein A chromatography runs were analyzed for detergent clearance and protein quality.

TABLE 2 | Properties of mAbs used in this study.

Product	IgG type	Mol wt. (kDa)	pI
mAb 1	IgG4	146.5	7.3
mAb 2	IgG1	151.6	9.0
mAb 3	IgG2	147.0	7.0

2.7.1 | Lab Scale Studies

Detergent clearance assessments were performed on three detergents C12-GLY10, C16-AO, and C11/15-sEO9 at lab scale in the presence of mAb 1, mAb 2, and mAb 3. Detergent stock solutions were added to the HCCF to achieve a final detergent concentration of 50-fold CMC. The HCCF material for all three product molecules was incubated with the three detergents for > 24 h at ambient temperature and processed through the protein A capture step. For the control run, the HCCF of three product molecules was maintained at ambient temperature for > 24 h without detergent.

The laboratory-scale protein A chromatography was performed using four MabSelect SuRe LX columns (1.6×20 cm) and product loaded to 35 mg/mL capacity for all purifications. Additional wash steps were performed between runs to mitigate against detergent carry-over. A 3CV 20% (v/v) ethanol wash step was followed by 3CV of equilibration buffer.

The product load and eluate streams from the protein A chromatography runs with detergents were collected and detergent concentrations measured to calculate the $\log_{10}$ clearance factors. Detergent clearance was calculated based on the amount present in the feed stream at the time of load. In addition, the detergent concentrations of waste streams were determined for the mAb 1 run to monitor the fate of the detergent and assess residual carry-over between runs. The eluted mainstreams were analyzed for product quality by size-exclusion chromatography (SEC-HPLC) and imaged capillary isoelectric focusing (icIEF) analysis.

2.7.2 | Pilot Scale Studies

Pilot-scale detergent clearance assessments were performed for C16-AO and C11/15-sEO9 with mAb 1. Detergent stock solutions were added to the HCCF to achieve a final detergent concentration of 1% w/w. The detergent was then mixed for 10 min followed by incubation for 60 min. The protein A chromatography step was performed using an OPUS pre-packed Praesto Jetted A50 column (10 cm diameter). The product and waste streams from the protein A capture step were sampled for detergent clearance analysis.

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2.8 | Multi-Criteria Decision Analysis

MCDA was performed using the virus inactivation (screening) data, CMC (% w/w) data, solubility study data, and storage conditions for each of the detergents.

Decision making was performed using a weighted sum model (WSM), shown in Equation (1). Where A_i is the score for detergent *i, j* is the criteria, *w* is the weight for the criteria *j*, a_{ij} is the score for detergent *i*, at criteria *j*.

$$A_i^{\mathrm{WSMscore}} = \sum_{j=1}^{n} w_j a_{ij}, \quad for \quad i = 1, 2, 3, \ldots, m. \tag{1}$$

The sum of the weighting must add up to a total of 1 across all criteria and is based on Equation (2), where *W* is the weighting portion for criteria *j* and the weighting of each criterion is w_j. The WSM weighting proportions applied for each criterion in the MCDA were 1:1:1:2 CMC (% w/w), Solubility, Storage Conditions, and Virus inactivation, respectively (Supporting Information S1: Table S.III).

$$w_j = \frac{W_j}{\sum W}. \tag{2}$$

The detergents that scored higher than TX-100 were taken forward for further testing.

3 | Results and Discussion

Sixteen detergents were identified that had the potential to be effective viral inactivation agents, focusing predominately on the non-ionic and zwitterionic class of detergent molecules. This study was performed in two stages.

3.1 | Stage One—Screening

3.1.1 | Solubility

To limit dilution of the HCCF during detergent addition, experimental studies were performed with the 16 detergents to determine if stock solutions could be prepared that result in no more than a 2%–5% volume increase after addition (Table 3). The target detergent stock solution concentrations were set to achieve a final HCCF concentration equivalent to between 5- and 50-fold the reported CMC value. For detergents that were not sufficiently soluble, either the percentage addition and HCCF dilution was increased or the final concentration (fold CMC) lowered.

Only 7 out of the 16 detergents (C12-EO8, C13-EO8, C16-AO, C11/15-sEO9, C12-GLY10, C18-GLY10, and C8/C10-GLY10) could be prepared at the stock solution concentrations required to achieve a final concentration of 50-fold CMC in the HCCF without exceeding the 2% dilution threshold.

3.1.2 | CMC Measurements

CMC values are usually measured in water, however, in the virus inactivation step the detergent is added to the HCCF which contains lipids, proteins, and other molecules that may interfere with CMC measurement. Therefore, it was necessary

TABLE 3 | A summary of the data used in the MCDA in stage one.

Detergent abbreviation	Solubility (% w/w addition)	Log_{10} reduction value (LRV) of PRV	Storage conditions	CMC (% w/w)*
C12-PO	2	≥ 3.7	Ambient	0.046
C10-SB	11	≥ 5.1	Ambient	1.1
CHAPSO	11	≥ 5.1	Ambient	0.16
C12-EO8	2	≥ 3.6	Ambient	0.012
C12-AO	4	≥ 4.1	5°C	0.31
C13-EO8	2	≥ 2.7	Ambient	0.031
C12-APAO	2	≥ 5.1	Ambient	0.050
C12-AA	2	≥ 4.1	Ambient	0.077
C12-APB	11	≥ 4.6	−20°C	0.076
C8-GLU	11	≥ 4.6	−20°C	1.1
C8/C10-GLY10	2	5.4	Ambient	0.042
C12-GLY10	2	5.3	Ambient	0.021
C18-GLY10	2	N/A	Ambient	0.014
C12-SUC	11	≥ 5.1	5°C	0.015
C16-AO	2	≥ 2.7	Ambient	0.002
C11/15-sEO9	2	≥ 2.6	Ambient	0.012
TX-100	2	≥ 2.6	Ambient	0.04

Note: CMC (%w/w)* was calculated using the formula: (experimental CMC × Mol. wt/1000) × 0.1. Data include the % w/w addition depending on the solubility, log reduction of PRV, storage conditions and % w/w addition depending on the CMC.

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to determine the experimental CMC for each detergent prepared with modified CCM. The reported CMC and the determined CMC values using the fluorescence method were comparable for most detergents (Supporting Information S1: Table S.IV). For instance, the reported CMC for TX-100 was 0.20–0.90 mM (supplier data), and the experimental CMC was 0.62 mM. However, for some detergents, the measured CMC was not close to the predicted CMC. For example, for C8/C10-GLY10 the reported value is 0.01 mM (supplier data), and the experimental value was 0.65 mM for the fluorescence method. No additional studies were performed to investigate any significant differences between the reported and experimental values.

3.1.3 | Virus Inactivation Screening Experiments

The viral inactivation capabilities of the 16 detergents were determined at $15 \pm 1°C$. The detergent concentrations evaluated in this study were between 5- and 50-fold the reported CMC values. Viral inactivation of PRV was determined following 60 min of incubation with large volume sampling, and the $\log_{10}$ reduction values (LRV) were calculated. All the detergents showed virus inactivation except C18-GLY10 (Table 3). The LRV achieved is linked to the cytotoxic effect of the detergent on the indicator cell line and the sample dilution factor required to perform the assay. The highest dilution factors were required for C13-EO8, C16-AO, and C11/15-sEO9 and the lowest dilutions for C8/C10-GLY10 and C12-GLY10 (data not shown).

3.1.4 | Multi-Criteria Decision Analysis

The highest scoring detergents following stage one were C12-EO8, C16-AO, and C11/15-sEO9 since these performed well in all of the criteria, reaching the maximum WSM score of 1. The next highest-scoring detergents were C13-EO8, C8/C10-GLY10, C12-GLY10, C12-PO, and C12-APAO with a WSM score of 0.9, comparable to TX-100. The remaining detergents scored lower than TX-100.

From the detergents with WSM score 1, C12-EO8 was excluded from further evaluation as it was not available in large quantities and the remaining two detergents C16-AO, and C11/15-sEO9 were shortlisted for stage two assessment (Supporting Information S1: Table S.III).

From the five detergents with WSM score 0.9, C13-EO8, C12-GLY10 were shortlisted for stage two assessment, C12-PO and C12-APAO were kept as back-up candidates and C8/C10-GLY10 was excluded as it exhibited high viscosity and might be challenging to use at the manufacturing scale. The detergents that scored lower than TX-100 were not further assessed.

3.2 | Stage Two—Assessment of Lead Candidates

3.2.1 | Virus Inactivation Kinetic Studies

The inactivation of X-MuLV and PRV was determined for the four lead candidates, C13-EO8, C12-GLY10, C16-AO, C11/15-sEO9, and TX-100 as the control. The kinetics of viral inactivation were monitored as a function of detergent concentration at $15 \pm 1°C$ between 0 and 60 min. TX-100 was included as a positive control in all assays.

Both large volume plating (LVP) and end point titration (EPT) virus titre assays were performed for all detergents, except C13-E08 for which only LVP was used for determining the virus log reduction.

Virus inactivation kinetics using the EPT are summarized in Figure 1.

Detergent treatment with TX-100 showed a $\geq 4.7 \pm 0.3$ $\log_{10}$ reduction of PRV and a 4.2 ± 0.3 $\log_{10}$ reduction of X-MuLV using LVP, following the 60-min incubation (Figure 2).

A 25-fold CMC concentration of C13-EO8 resulted in complete viral inactivation of PRV within 60 min of incubation resulting in an LRV of $\geq 5.2 \pm 0.3$ $\log_{10}$ using LVP. A 50-fold CMC concentration of C13-EO8 resulted in complete viral inactivation for X-MuLV within 10 min of incubation using LVP resulting in a $\geq 3.4 \pm 0.3$ $\log_{10}$ virus reduction. However, C13-EO8 was not available in a suitable reagent grade and considered as a

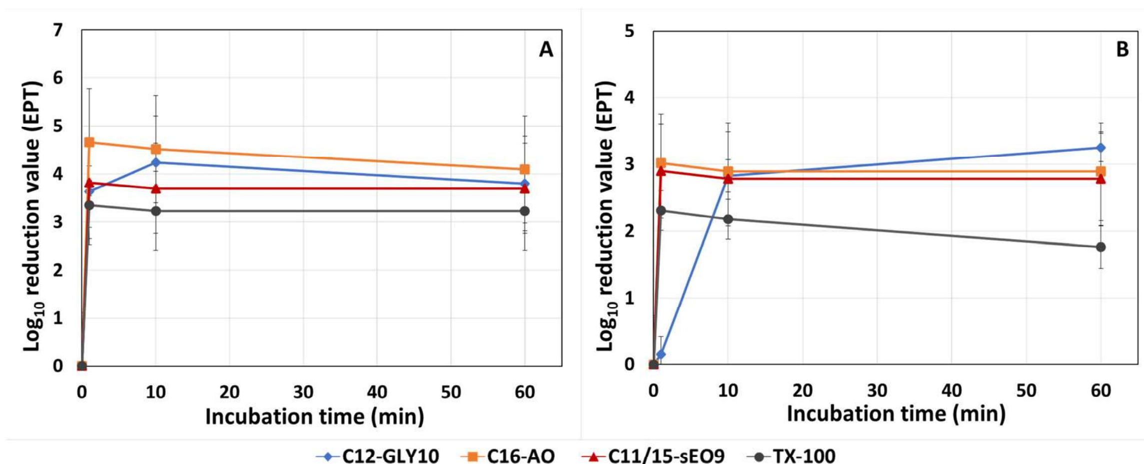

FIGURE 1 | Virus inactivation kinetics for PRV (A) and X-MuLV (B) in the presence of C12-GLY10, C16-AO, C11/15-sEO9 at 25-fold CMC, and TX-100 control based on endpoint titration.

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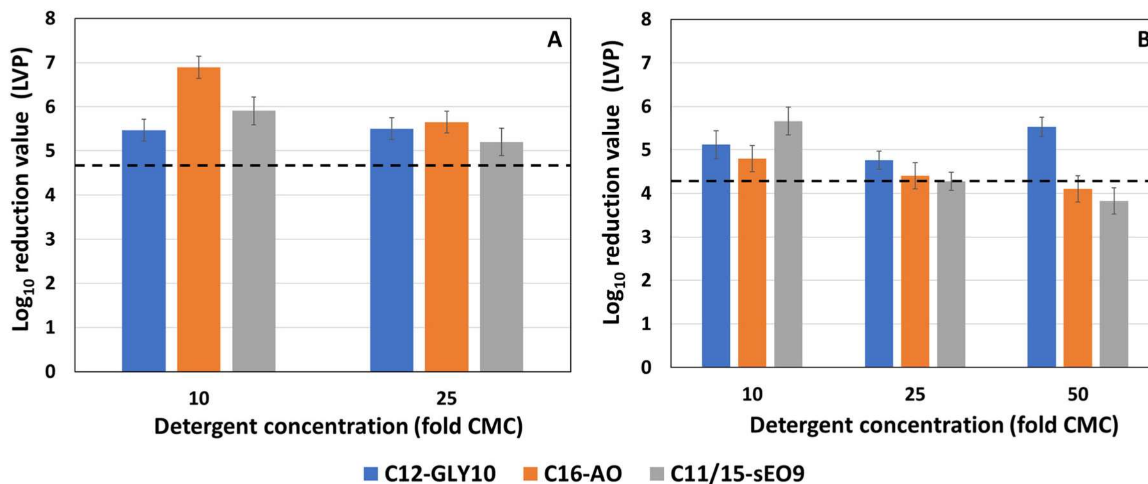

FIGURE 2 | Effect of detergent concentration on the virus inactivation of PRV (A) and X-MuLV (B) on incubation for 60 min.

potential back-up candidate only. No additional data were generated for this candidate in stage two (Supporting Information S1: Table S.V).

A virus reduction of 5.5 ± 0.3 $\log_{10}$ was observed using LVP for PRV at 25-fold CMC concentration of C12-GLY10 after incubating for 60 min. At this concentration, C12-GLY10 also showed good virus inactivation kinetics with X-MuLV, and a virus reduction of $\geq 4.8 \pm 0.2$ $\log_{10}$ was achieved after incubating for 60 min. Thus, the 25-fold CMC concentration of C12-GLY10 showed acceptable virus inactivation of ≥ 4 $\log_{10}$ for X-MuLV and PRV (Figure 2).

A 25-fold CMC concentration of C16-AO resulted in complete viral inactivation of PRV within 10 min of incubation and this was maintained at 60 min, resulting in an LRV of 5.7 ± 0.3 $\log_{10}$ reduction (LVP). Similarly, after 10 min of exposure to 25-fold CMC concentration of C16-AO, the virus titer was reduced to below detectable levels (LVP), to a value of $\geq 4.4 \pm 0.3$ $\log_{10}$ which was maintained between 10 and 60 min (Figure 2).

A 25-fold CMC concentration of C11/15-sEO9 resulted in complete viral inactivation of PRV within 10 min of incubation resulting in an LRV of 5.7 ± 0.3 $\log_{10}$ (LVP). At this concentration, C11/15-sEO9 resulted in complete viral inactivation of X-MuLV resulting in a $\geq 4.3 \pm 0.2$ $\log_{10}$ of viral inactivation within 10 min of incubation. C11/15-sEO9 shows effective inactivation against PRV and X-MuLV at 25-fold CMC concentration (Figure 2).

3.2.2 | Detergent Clearance Considerations

Based on the PRI risk assessment in-process testing should be undertaken for the detergent additives. The values for the PDE IV and PRI dose were used to calculate the desired $\log_{10}$ clearance factor as well as the detergent assay limit of quantification (LoQ) required to demonstrate clearance. The desired $\log_{10}$ clearance required for C12-GLY10, C16-AO, and C11/15-sEO9 were 3.8, 2.5, and 3.3, respectively. The required detergent assay LoQ values were 1666, 467, and 1666 μg/L for C12-GLY10, C16-AO, and C11/15-sEO9, respectively. The LoQ values for C12-GLY10, C16-AO, and C11/15-sEO9 assays were 400, 20, and 150 μg/L, respectively which are considerably lower than required making these assays suitable to analyze in-process samples.

3.2.3 | Detergent Clearance Assessment at Laboratory Scale

Key process step performance indicators assessed in this study included turbidity of process intermediates, eluate pH, elution volume and step yield, as well as equipment and column performance indicators such as pressure (data not shown).

The performance indicators were comparable between chromatography runs with control feed streams, without detergents, and runs with C11/15-sEO9 and C16-AO-treated feed streams. The runs with C12-GLY10 treated feed streams matched the controls on all performance indicators with the exception of the HCCF turbidity and column pressure. An increase in turbidity was observed upon the > 24 h incubation of C12-GLY10 with the HCCF which remained unchanged after 0.22 μm filtration. A transient increase in column inlet pressure, most likely caused by the turbidity of the feed stream, was observed during the protein A loading phase; the column inlet pressure was comparable to the control after the first wash phase.

Based on the detergent assay, only 5%–15% of the initial C12-GLY10 remained in the feed stream, suggesting that the detergent was likely degraded in the HCCF. A similar observation was made by Chen et al. (2020) with the hydrolysis of the ester bond linking the hydrophilic and hydrophobic moieties of polysorbate 80 in HCCF. The subsequent formation of C12 lipid micro particles may have resulted in the turbidity increase, but this was not confirmed. Further work is required to elucidate the mechanism leading to the elevated turbidity. C12-GLY10 was therefore was not evaluated further and the pilot scale runs were performed only with C16-AO and C11/15-sEO9.

C12-GLY10, C16-AO, and C11/15-sEO9 had no impact on the product quality and HCP clearance with mAb 1, mAb 2, and mAb 3 following protein A chromatography. No changes were

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observed in the distribution of the acidic and basic variants or the concentration of aggregates and fragments in the presence of C12-GLY10, C16-AO, or C11/15-sEO9 (Supporting Information S1: Table S.VII).

All the process intermediates from the protein A chromatography runs with detergents for mAb 1 were collected and the detergent concentrations determined. The mass balance for both C16-AO and C11/15-sEO9 detergents were within acceptable ranges and the results were used to determine the fates of these two detergents during the chromatography step. In both cases, the bulk of the detergent was present in the load flowthrough, no accumulation on the resin was observed and residual detergent was readily washed off the resin during the product wash steps (Supporting Information S1: Table S.VI). The detergent concentration was below the limit of detection for the additional wash step performed after the elution of the product, indicating negligible carry-over between runs.

Table 4 summarizes the log_{10} clearance results of 50-fold CMC concentration of C12-GLY10, C16-AO, and C11/15-sEO9 obtained with the lab-scale protein A chromatography step. The detergent clearance values for all three detergents with all three mAbs were found to be higher than the desired log_{10} reduction derived from the toxicological assessment.

3.2.4 | Detergent Clearance Assessment at Pilot Scale

C16-AO and C11/15-sEO9 were used for the virus inactivation process in two pilot batches. The detergent addition did not have any impact on the protein A chromatography step performance. Compared to the control runs, no differences in feed stream turbidity, column pressure or normalized elution volumes were observed.

Product quality analysis of protein A eluates showed that the addition of C16-AO or C11/15-sEO9 had no impact on the fragment, aggregate or charge variant attributes of mAb 1 (Supporting Information S1: Table S.VII).

The detergent clearance values for the two detergents with mAb 1 were found to be higher than the desired log_{10} reduction derived from the toxicological assessment. The detergent clearance value for C16-AO was 4.0 log_{10} compared to the desired log reduction of 2.8 log_{10}. Similarly, the detergent clearance value for C11/15-sEO9 was 3.9 log_{10} compared to the desired log reduction of 3.6 log_{10} derived from the toxicological assessment (Supporting Information S1: Table S.VI).

TABLE 4 | Detergent clearance (log_{10}) values through the protein A step for C12-GLY10, C16-AO, and C11/15-sEO9 at lab scale.

Molecule	C12-GLY10	C16-AO	C11/15-sEO9
mAb 1	5.1	4.7	5.5
mAb 2	4.7	4.3	5.9
mAb 3	5.2	4.8	5.7
Desired log_{10} reduction	3.3	2.5	3.8

Note: Detergents were added to the HCCF at an initial concentration of 50-fold of their CMC values.

Finally, there were no issues in handling the detergents at manufacturing scale resulting in the successful scale-up of the C16-AO and C11/15-sEO9 virus inactivation processes.

4 | Conclusions

Two detergents C16-AO and C11/15-sEO9, were identified as viable alternatives to TX-100 for use in protein therapeutic production processes for inactivating enveloped viruses. The zwitterionic detergent C16-AO and non-ionic detergent C11/15-sEO9, are as effective as TX-100 for the inactivation of enveloped viruses but are more eco-friendly based on toxicology assessment. In addition, C13-EO8 exhibited good viral inactivation kinetics and warrants further evaluation. Importantly, all three agents are not listed on the ECHA's candidate list of SVHC or the REACH Annex III inventory and are of low concern according to the US EPA Safer Chemical Ingredients.

A two-step approach was implemented to screen 16 potential candidates and select leads for detailed assessment. During stage one screening, data generated on solubility, CMC, and viral inactivation were inputted into MCDA along with storage conditions resulting in the identification of eight candidates—C12-EO8, C13-EO8, C8/C10-GLY10, C12-GLY10, C12-PO, C12-APAO, C16-AO, and C11/15-sEO9—that were better than or similar to TX-100 based on their WSM score.

In stage two, four lead candidates—C13-EO8, C12-GLY10, C16-AO, and C11/15-sEO9—were assessed for viral inactivation. Overall, total PRV and X-MuLV inactivation was achieved with 25-fold CMC concentration of C12-GLY10, C16-AO, and C11/15-sEO9 at 60 min incubation which was comparable to that observed with TX-100 prepared as per Lonza protocol. In addition, 25-fold and 50-fold CMC concentration of C13-EO8 achieved good viral inactivation with PRV and X-MuLV respectively, but further evaluation was not feasible due to supply issues.

The toxicology, process performance, product quality, and clearance of the three detergents C12-GLY10, C16-AO, and C11/15-sEO9 was assessed. At laboratory scale, C16-AO and C11/15-sEO9 addition to the HCCF showed no impact on process performance, product quality, and HCP clearance. The Protein A step with all the three mAbs provided detergent clearance which was higher than the desired log_{10} reduction derived from the toxicological assessment for all three detergents. C12-GLY10 exhibited high turbidity after addition to the HCCF at laboratory scale and was not evaluated at pilot scale.

At pilot scale, C16-AO and C11/15-sEO9 addition had no impact on overall process performance or product quality, Both detergents were cleared to non-detectable levels by the DSP resulting in detergent clearance values higher than the desired log_{10} reduction values derived from the toxicological assessment.

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All these findings suggest that C16-AO and C11/15-sEO9 are viable eco-friendly alternatives to TX-100; Zwitterionic and non-ionic detergent options have been identified that can be selected based on product compatibility requirements. Biomanufacturing grade materials for C16-AO and C11/15-sEO9 are available with trade names Deviron C16 (ISO9001) and Deviron 13-S9 (IPEC-PQG GMP) respectively. In addition, C13-EO8 demonstrated good viral inactivation capabilities and warrants further investigation in detergent clearance and other processes. C12-GLY10 also showed promise but further work is required to optimize concentrations and address the turbidity issues.

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Conflicts of Interest

The authors declare no conflicts of interest.

Data Availability Statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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