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Impact of multiple re-use of anion-exchange chromatography media on virus removal☆

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Abstract

We evaluated viral clearance in multiply-cycled anion-exchange media run in flow-through mode. We found that anion-exchange columns do not lose viral clearance capacity after extensive re-use, if they are cleaned with recommended buffers that do not chemically degrade the media. In contrast, anion-exchange (AEX) columns that are not cleaned or are cleaned with buffers that chemically degrade the media lost viral clearance capacity after extended use. In these cases, other performance attributes that changed at the same time were increased band spreading, decreased DNA clearance and accumulating backpressure that prevented re-use past 80–120 cycles. Thus, our data suggests that flow through mode anion-exchange columns that are cleaned with recommended cleaning buffers, and periodically monitored for band spreading, DNA clearance and/or backpressure need not be re-evaluated for viral clearance at the end of the validated media lifetime.

Keywords: Viral clearance; Biotechnology; Bioprocessing; Anion-exchange media; Monoclonal antibodies; Chromatography media lifetime

1. Introduction

Because of process economics, chromatography media is extensively re-used in biopharmaceutical manufacture, often more than 100 times [1,2]. Multiple re-use of chromatography media poses a theoretical safety risk because declining performance of the media may lead to diminished virus removal [3]. For example, protein or lipid build-up can interfere with the performance of media, particularly when improperly sanitized [4–6]. Ligand loss or degradation of the matrix support can also reduce the capacity of a resin [7–12]. The impact of media degradation on viral clearance will vary with media type. For example, protein A media specifically binds antibodies (Abs), while endogenous retroviruses present in cell culture harvests flow largely uninhibited through the column [13,14]. The aforementioned mechanisms of decay are not predicted to impact the uninhibited flow of viruses through the column during loading, and this prediction was verified in a study examining protein A sepharose lifetime and virus clearance [8].

In contrast, anion-exchange (AEX) columns are believed to remove negatively charged viruses from in process intermediates by binding them more avidly than positively charged drug substances like monoclonal antibodies [13,15]. Gene therapy and vaccine manufacturing schemes often use AEX and other resins with salt elutions to separate vaccine or therapeutic viruses from differently charged process impurities [16–25]. When run at analytical scale with proper cleaning, many types of media can be used up to 1000 cycles [26,27]. When run on a preparative scale, protein and other impurity build-up can limit media lifetime and interferes with binding and retention time [5,6,28]. Other potential mechanisms of decay that have been shown to impact binding and

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retention times are gradual losses of functional groups and matrix hydrolysis caused by harsh or inappropriate cleaning buffers [10–12,28–31]. Fouling, matrix disintegration and ligand loss could eliminate potential binding sites for viruses, conceivably impacting viral clearance capacity.

A demonstration of viral safety is required by regulatory agencies world-wide for registration or investigational new drug (IND) use of biotechnology drug products produced by mammalian cell cultures [3,32]. The viral safety demonstration includes screening of cell banks and cell culture harvests for adventitious viruses, virus removal validation studies of the drug substance purification process and a quantification of endogenous retrovirus levels in cell culture harvests. In the validation studies, the virus $\log_{10}$ reduction value (LRV) of individual purification unit operations, such as chromatography or nanofiltration, is measured in scaled-down studies that mimic the unit operation using model viruses and in process intermediates. If applicable, an assessment of the impact of extended processing of column unit operations on virus clearance is recommended by the International Conference on Harmonization (ICH) [2,3].

Two safety assessment approaches have been proposed to satisfy the ICH recommendation. The most conservative (and expensive) approach is to perform small-scale virus removal studies using intermediates from each new process, using both new and re-used column media. A second approach is performing virus removal validation studies on new media only and then monitoring during production chromatography performance attributes such as product step yield or eluate impurity content (protein A, DNA, etc.) predicted to decay prior to virus clearance. This approach requires the identification of such a performance attribute, but obviates the need for measuring virus $\log_{10}$ reduction value by used media on a product-by-product basis. Recently, this approach was evaluated using lab scale murine hybridoma cell culture concentrates and protein A sepharose fast flow as a model protein A resin [8]. These studies identified Ab step yield and breakthrough as performance attributes that decay prior to retrovirus LRV when protein A sepharose fast flow media is multiply-cycled.

Performance attributes often monitored during flow-through anion-exchange chromatography include impurity removal (DNA, small molecule or protein), step yield, packing integrity by frontal analysis of chromatogram profiles or band spreading by theoretical plates analysis [1,2,33,34]. The step yield of a flow through unit operation is likely to change only in cases of unexpected increased product binding due to resin chemistry changes. Forms of anion-exchange resin degradation (fouling, compaction, ligand loss) are more likely to be detected by monitoring other parameters such as greater or more uneven band spreading, changes in chromatogram profiles or decreased impurity removal. Thus, it would be of interest to determine which of these performance attribute changes prior to or at the same time as virus LRV so they could be monitored as surrogates for virus LRV in extensively cycled AEX columns.

In this report, we used commercial process intermediates in appropriately scaled down studies to cycle anion-exchange resins from three manufacturers up to 200 cycles or until resin failure. We used three different cleaning strategies and monitored backpressure, band spreading, frontal analysis, DNA removal, step yield and virus removal in an effort identify key stability indicating performance attributes for flow through mode anion-exchange chromatography.

2. Experimental

2.1. Chromatography media and feedstocks

Q-Sepharose fast flow (lot no. 296307) was purchased from Amersham (Uppsala, Sweden). Q-Ceramic Hyper D (lot no. 8066) was a kind gift from B. Schwartz and M. Mahard (BioSepra SA, Cergy-St. Christophe, France; [35]). Toyopearl super Q-650M (lot no. 65QAM99B) was purchased from TosoHaas (Tokyo, Japan; [36]). Purified monoclonal antibody (mAb) was produced at Genentech using proprietary processes.

2.2. Chromatography system, buffers and run cycles

Small-scale chromatography models were designed to be realistically representative of a commercial process, but at the same time, near or beyond typical extreme limits of key process scale parameters such as protein load, load quality, linear flow rate and bed height. Extreme limit conditions were chosen to challenge the stability of virus clearance by anion-exchange chromatography, testing its robustness in this context.

AEX media was packed into a 0.67 cm diameter Omnifit glass analytical chromatography housing unit (Omnifit, Cambridge, UK, 3.9 ml resin, 11 cm bed height). The columns were integrated into an AKTA explorer 100 chromatography system run by UNICORN v3.21.02 software (Amersham). The system was programmed to repeatedly run the following chromatography cycle: equilibrate at 40 column volumes (CVs)/h with 3 CVs of 25 mM Tris, 7.5 mS/cm (∼50 mM) NaCl, pH 8.0; flow mAb in same buffer adjusted to the same conductivity and pH through the column at 40 CVs/h. Sufficient mAb was loaded to pass 50 mg mAb through each ml of resin. The load consisted of a purified proprietary mAb intermediate (2 mg/ml; Genentech) spiked with 2 ug/ml mouse genomic DNA. The column was then washed with 3 column volumes of equilibration buffer at 40 CVs/h; regenerated at 40 CVs/h with 2 CVs of 250 mM Tris, 2 M NaCl, pH 8.0; and finally cleaned at 40 CVs/h with 2CVs with either 0.5 M NaOH, 100 mM HCl or nothing. All steps were performed in down-flow except for a 15 s up-flow pulse during the cleaning buffer wash designed to dislodge material trapped in the top frit. The system was programmed to start each run by running a cycle where the mAb/DNA load was spiked with $\sim 5 \times 10^4$ tissue culture infectious doses $(TCID)_{50}$/ml xenotrophic murine leukemia virus (X-MuLV; Bioreliance,

Rockville, MD) and the entire mAb flow through was collected for analysis. When loaded at pH 8.0 and conductivity of 7.5 mS/cm, the mAb (pI = 9) flows through the Q-columns unbound, while X-MuLV and DNA bind.

The program then cycled through the following routine two to seven times: eight cycles without mAb collection; one blank run cycle where no protein was loaded but a equilibration buffer pass-through fraction was collected; one X-MuLV spiked cycle where the mAb was collected (>100 mAU_{280} above background). To achieve an overall ~200 cycles for each resin, up to six individual runs were performed for each resin/cleaning buffer combination. In cases where significant aeration problems arose, as determined by UV absorbance spikes, the columns were re-packed with as much of the original resin as possible, maintaining near equivalent bed heights. Experiments where 200 cycles could not be achieved due to persistent backpressure (>1 mPA) were terminated early. Fraction volumes, buffer types, resin type and bed height, collection cycles, and run lengths were recorded for each experiment on run sheets. The AKTA system continuously recorded conductivity, flow, UV absorbance, backpressure, and valve position data.

Measurement of the number of theoretical plates of each column was performed approximately every 50 cycles and at the start and end of each experiment. Theoretical plates are generally considered to be a quantitative measure of column quality, with higher numbers correlating with increased column performance [34]. Each theoretical plate measurement consisted of a 6.2 CVs wash with deionized water (dH_2O), 25 μl pulse of 2% acetone in dH_2O, followed by a 4.5 CVs wash with dH_2O. To amplify the effect of resin decay on theoretical plate number, the flow rate was slightly above the flow rates using in the cycling experiments, 47 CVs/h. Asymmetry of the acetone A_{280} peak and number of theoretical plates of the column were calculated using the UNICORN software that runs the AKTA system (Fig. 1A). The mathematical formula for number of theoretical plates (N) is $N = 5.54 \times (V_R/W_h)^2$, where V_R is the peak retention time in minutes and W_h is the peak width at half height in minutes. The mathematical formula for asymmetry is asymmetry = width $B \div$ width A, where A and B are the partial peak widths measured at 10% of the peak height, with A representing the leading part of the peak and B the trailing part of the peak. A peak with no asymmetry has a value of 1.0, a peak with a left skew has a value less than 1.0, while a peak with a right skew has a value greater than 1.0.

Analysis of the mAb-loading front was performed by calculating the first derivative of the A_{280} absorbance curve. Asymmetry and half-maximal width of the first derivative curve peak was calculated using the UNICORN software that runs the AKTA system (Fig. 1B).

At the end of each experiment, the anion-exchange resins were recovered and re-run one additional time under similar conditions, except simian virus 40 (SV40) and minute virus of mice (MVM) were used to spike the mAb instead of X-MuLV and mouse genomic DNA. The bed heights were held constant at 9 cm due to resin loss during unpacking and repacking, and because of compaction of some resins due to multiple cycling with non-recommended cleaning strategies (e.g. no cleaning, HCl cleaning). As a comparison, fresh anion-exchange resins were also run under identical conditions, also with 9 cm bed heights.

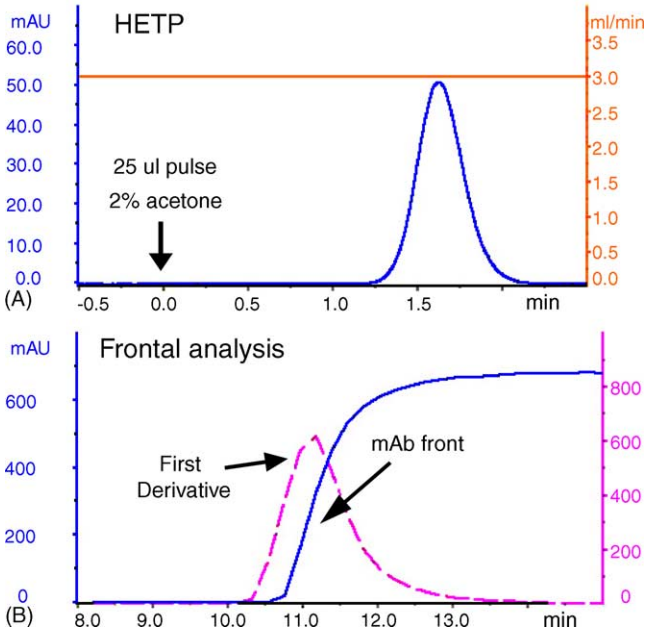


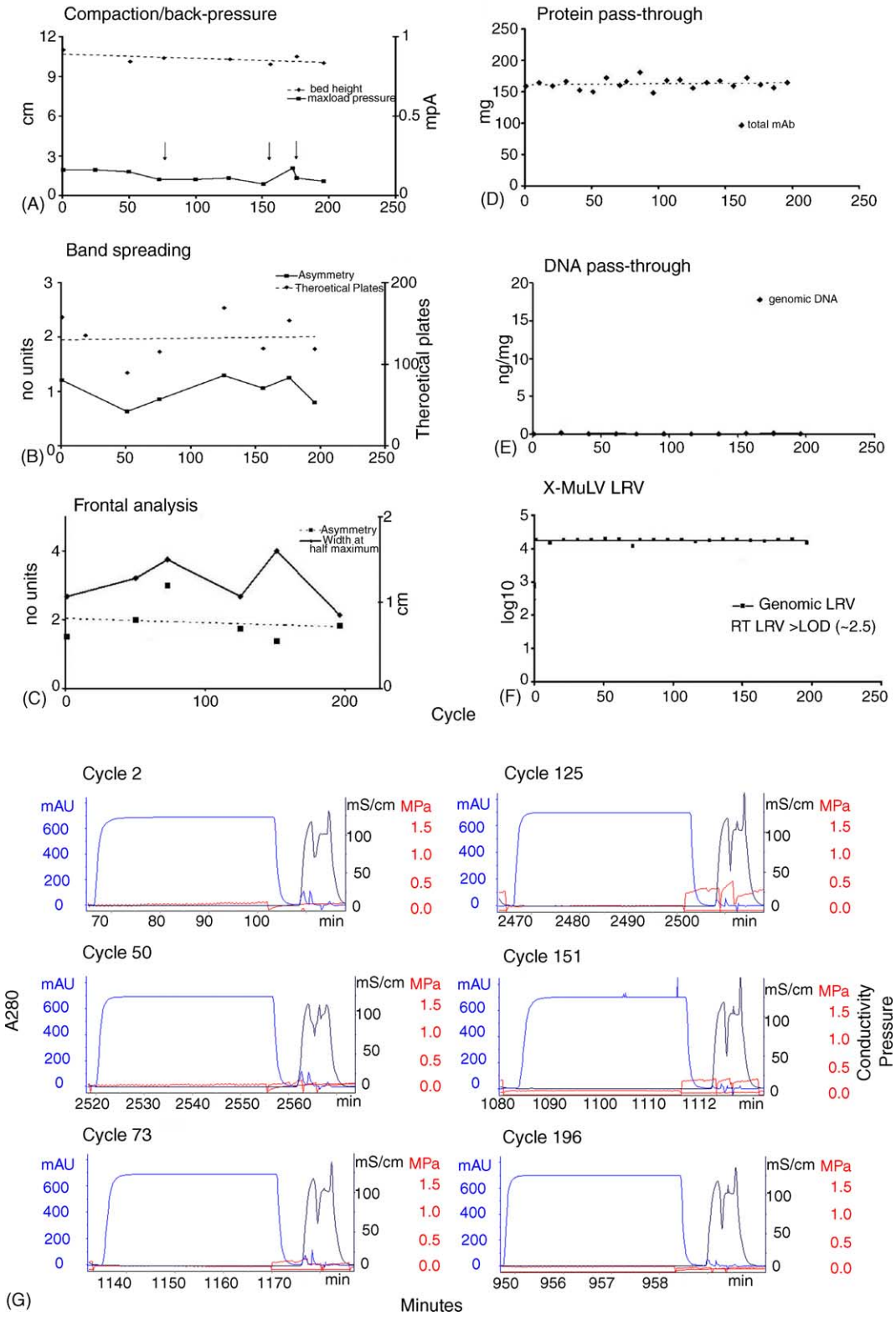
Fig. 1. Theoretical plates and frontal analysis of fresh Q-Sepharose resin run under the conditions described in this study. (A) Band spreading and asymmetry of a 2% acetone pulse. (B) Chromatogram of A_{280} of a mAb front and the first derivative of the A_{280} curve.

2.3. Q-PCR assays

Quantitative TaqMan fluorogenic 5′ nuclease product-enhanced reverse transcriptase (Q-PERT) assays were performed as described [14,37,38]. Mouse genomic DNA [8], X-MuLV [39], SV40 [40,41] and MVM [39,42] clearance was measured using Q-PCR assays as described.

2.4. Genomic DNA preparation

Organs were harvested from mice (kidney, liver, lung, heart, brain, thymus, and ovaries or testes) and homogenized in 100 mM NaCl, 10 mM Tris, 25 mM EDTA, pH 8.0. Proteinase K (0.1 mg/ml) and sodium dodecyl sulfate (SDS, 0.5%) were added and the mixture was digested overnight at 50 °C. The digestion mix was extracted for 1 h at 20–25 °C with phenol:choloroform:isoamyl alcohol (25:24:1 volume ratio). The DNA was precipitated from the aqueous phase after extraction by the addition of NaOAc to 0.3 M and two volumes of ethanol. After centrifugation for 5′ at 10,000 × g, the DNA pellet was resuspended at ~200 ug/ml in TE 8 buffer (10 mM Tris, 1 mM EDTA, pH 8.0). All mice were sacrificed under FDA animal care and use committee protocols.


Compaction/back-pressure
cm
mpA
bed height
maxload pressure
(A)
Protein pass-through
mg
total mAb
(D)
Band spreading
no units
Theroetical plates
Asymmetry
Theroetical Plates
(B)
DNA pass-through
ng/mg
genomic DNA
(E)
Frontal analysis
no units
cm
Asymmetry
Width at half maximum
(C)
X-MuLV LRV
log10
Genomic LRV
RT LRV >LOD (~2.5)
(F)
Cycle
Cycle 2
Cycle 125
Cycle 50
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Cycle 73
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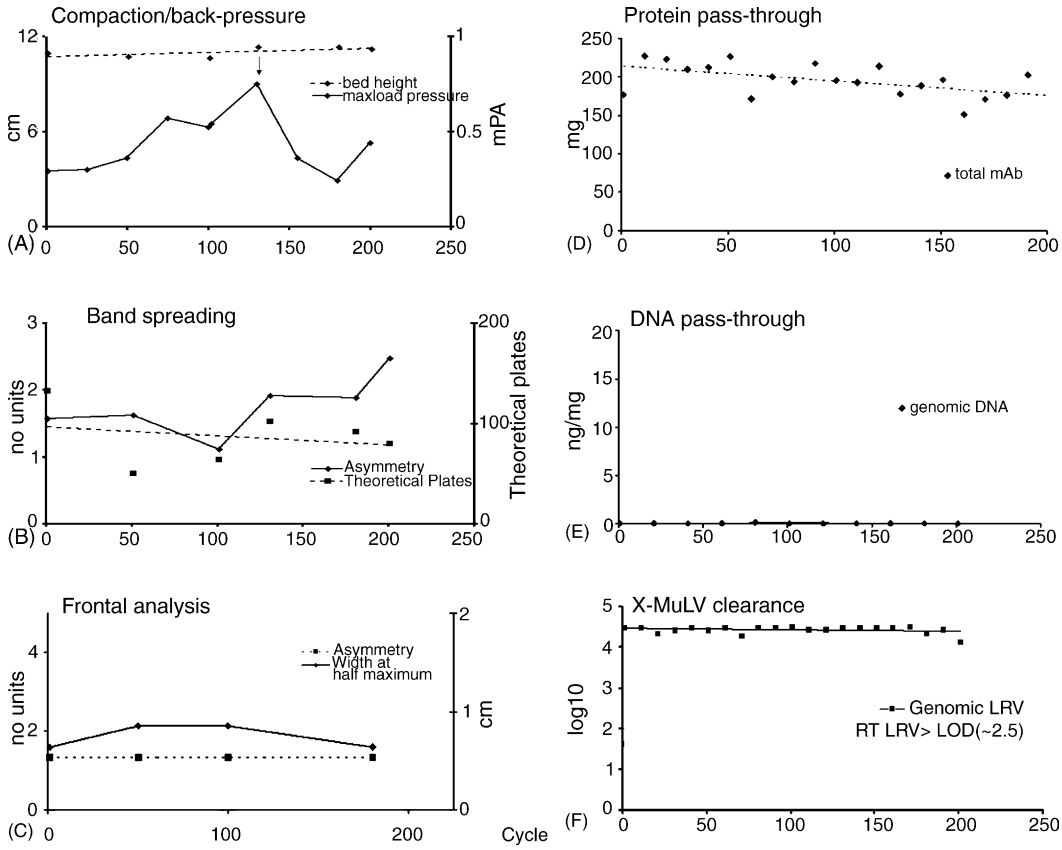

Fig. 3. Q-Ceramic Hyper D column run through 201 cycles of product pass-through/regeneration/cleaning with 0.5 M NaOH cleaning buffer. The experiment consisted of five separate runs captured in independent run files (cycles 1–50, 51–100, 101–130, 131–180, 181–201). The column was repacked after cycle 130. (A–F) Panel identifications and use of lines and symbols are the same as Fig. 2.

3. Results and discussion

3.1. Multiple cycling of anion-exchange resins cleaned with recommended cleaning buffers

NaOH-based solutions are commonly considered to be effective cleaning buffers which do not excessively impact the stability of many ion exchange chromatographic resins [9,12,35,43,44]. To investigate the stability of viral clearance by anion-exchange chromatography, media were subjected to ∼200 sequential load/NaOH cleaning cycles with minimal manipulation or storage between cycles. Resins with different matrix chemistries (agarose-based Q-Sepharose fast flow, polystyrene/ceramic-based Q-Ceramic Hyper D and vinyl polymer-based Toyopearl super Q-650M) were chosen to broaden the range of resin stability profiles included in the study [29]. The model load was a monoclonal antibody spiked with mouse genomic DNA as a model process impurity and X-MuLV, a model contaminating murine retrovirus. A variety of performance attributes were measured over extended cycling and are plotted in Figs. 2–6: (1) retrovirus clearance using two orthogonal Q-PCR based assays; (2) DNA clearance; (3) step yield; (4) band spreading of pulse of 25 μl 2% acetone; (5) shape of the UV absorbance front at ini-

Fig. 2. Q-Sepharose fast flow column run through 196 cycles of product pass-through/regeneration/cleaning with 0.5 M NaOH cleaning buffer. The experiment consisted of six separate runs captured in independent run files (cycles 1–50, 51–75, 76–125, 126–155, 156–175, 176–196). The column was repacked after cycles 75, 155, and 175. (A) Bed height/compaction (diamonds and dashed lines) and maximum backpressure during mAb pass-through (squares and solid lines) was measured before each run and at the end of the experiment. Arrows indicate re-packing between cycles. (B) Band spreading was measured from the A_{280} curve of a 25 μl pulse of 2% acetone. Diamonds and dashed lines represent theoretical plates calculated from the acetone peak; squares and solid lines are the asymmetry of the acetone peak. (C) Frontal analysis was performed on the protein front at the start of mAb pass-through. Squares and dashed lines are the asymmetry of the curve generated by taking the first derivative of the A_{280} protein front curve. Diamonds and solid lines are the peak width at half of the maximal height of the same curve. (D) Diamonds represent total protein content of mAb collected during product pass through, measured every 10 cycles. The dashed line indicates the linear trend of total protein pass-through. (E) Diamonds and solid lines represent DNA contamination in mAb collected during product pass through. The DNA content in ng/mg of total protein was measured every 20 cycles. (F) Squares represent clearance of X-MuLV measured every 10th cycle. The solid line is the linear trend of the LRV. Clearance of RT activity in this experiment exceeded the measurement capacity of the assay (>2–3 $\log_{10}$). (G) Representative chromatograms from throughout the run. Blue line is A_{280} absorbance, black line is conductivity, red line is column backpressure both during buffer pass-through and during mAb pass-through.

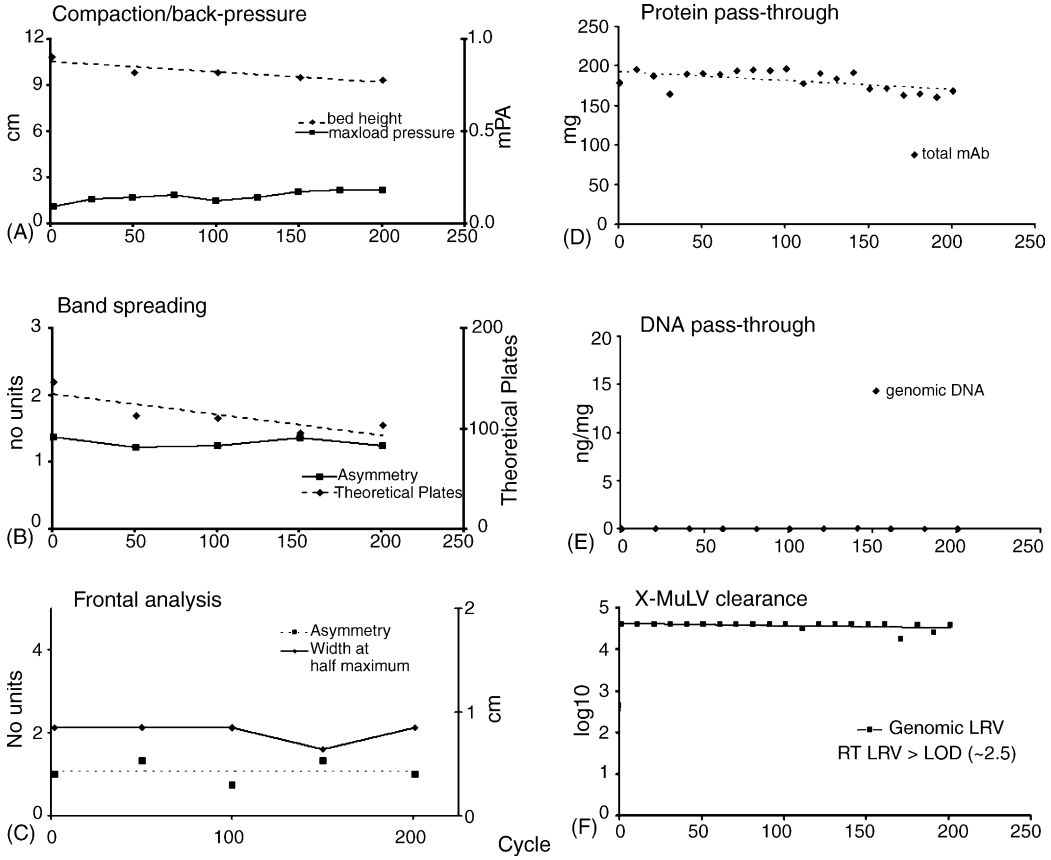

Fig. 4. Toyopearl super Q-650M column run through 201 cycles of product pass-through/regeneration/cleaning with 0.5 M NaOH cleaning buffer. The experiment consisted of four separate runs captured in independent run files (cycles 1–50, 51–100, 101–150, 151–201). The column was not repacked. (A–F) Panel identifications and use of lines and symbols are the same as Fig. 2.

tial mAb loading; (6) column backpressure. Representative chromatogram profiles from the Q-Sepharose fast flow experiments are shown in sub Figs. 2G, 5G and 6G. Resins were recovered at the end of each experiment, repacked into new chromatography housing units and compared with naïve resins for their capacity to clear two additional viruses, SV40 and MVM (Table 1).

In the case of all three resins, each property monitored in this study remained stable for the duration of 200 cycles, including clearance of X-MuLV. The columns needed re-packing only in cases of aeration problems unrelated to the column resin. LRV of X-MuLV, measured with a sensitive genome specific Q-PCR assay, was greater than 4.5 $\log_{10}$, with no one resin type appearing to possess an advantage over the other in this direct comparison (Figs. 2F, 3F and 4F). In two out of three cases, the fitted curve of the LRV data had a near zero or positive slope. In the third case, Q-Sepharose fast flow (QSFF), the slope was –0.0037 $\log_{10}$/cycle, consistent with a viral clearance capacity loss of 1 $\log_{10}$ for every ~300 cycles. This level of a change would only be considered to be significant from a regulatory standpoint if a QSFF column was actually used for 300 cycles in routine manufacturing. Resins from these experiments were recovered and tested for their clearance capacity of two additional viruses, SV40 and

Table 1
Clearance of SV40 and MVM of new and re-used anion-exchange resins

Resin	$\log_{10}$ reduction		Protein recovery (%)
	MMV	SV 40	
Q-Sepharose fast flow			
Naïve	≥5.12 ± 0.21	≥4.28 ± 0.14	98
Re-use			
198 cycles; 0.5 M NaOH	≥5.06 ± 0.07	≥4.41 ± 0.08	97
124 cycles; 0.1 M HCl	4.15 ± 0.13	3.33 ± 0.33	101
84 cycles; no cleaning	4.45 ± 0.13	3.96 ± 0.08	98
Q-Ceramic Hyper D			
Naïve	≥5.22 ± 0.02	≥4.67 ± 0.12	103
Re-use			
203 cycles; 0.5 M NaOH	≥5.17 ± 0.13	≥4.52 ± 0.02	102
Toyopearl super Q-650 M			
Naïve	3.49 ± 0.19	4.70 ± 0.30	100
Re-use			
203 cycles; 0.5 M NaOH	3.67 ± 0.25	≥4.81 ± 0.09	102

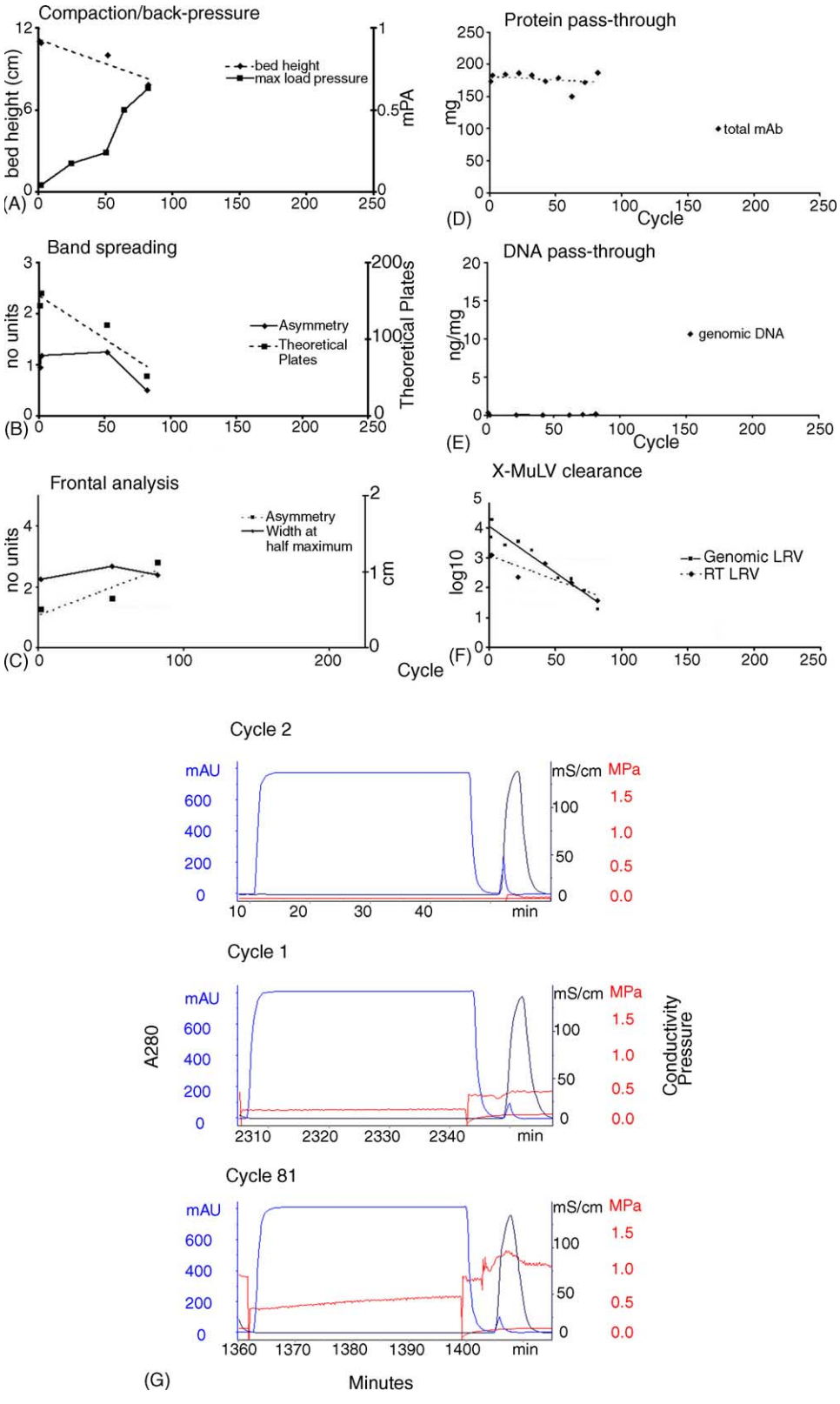

Fig. 5. Q-Sepharose fast flow column run through 82 cycles of product pass-through/regeneration with no cleaning step. The experiment consisted of three separate runs captured in independent run files (cycles 1, 2–51, 52–82). The column was repacked after cycle 1 due to aeration problems. (A–G) Panel identifications and use of lines and symbols are the same as Fig. 2 except in (F) where diamonds represent clearance of RT activity measured every 20th cycle and the dashed line is the linear trend of the RT LRV.

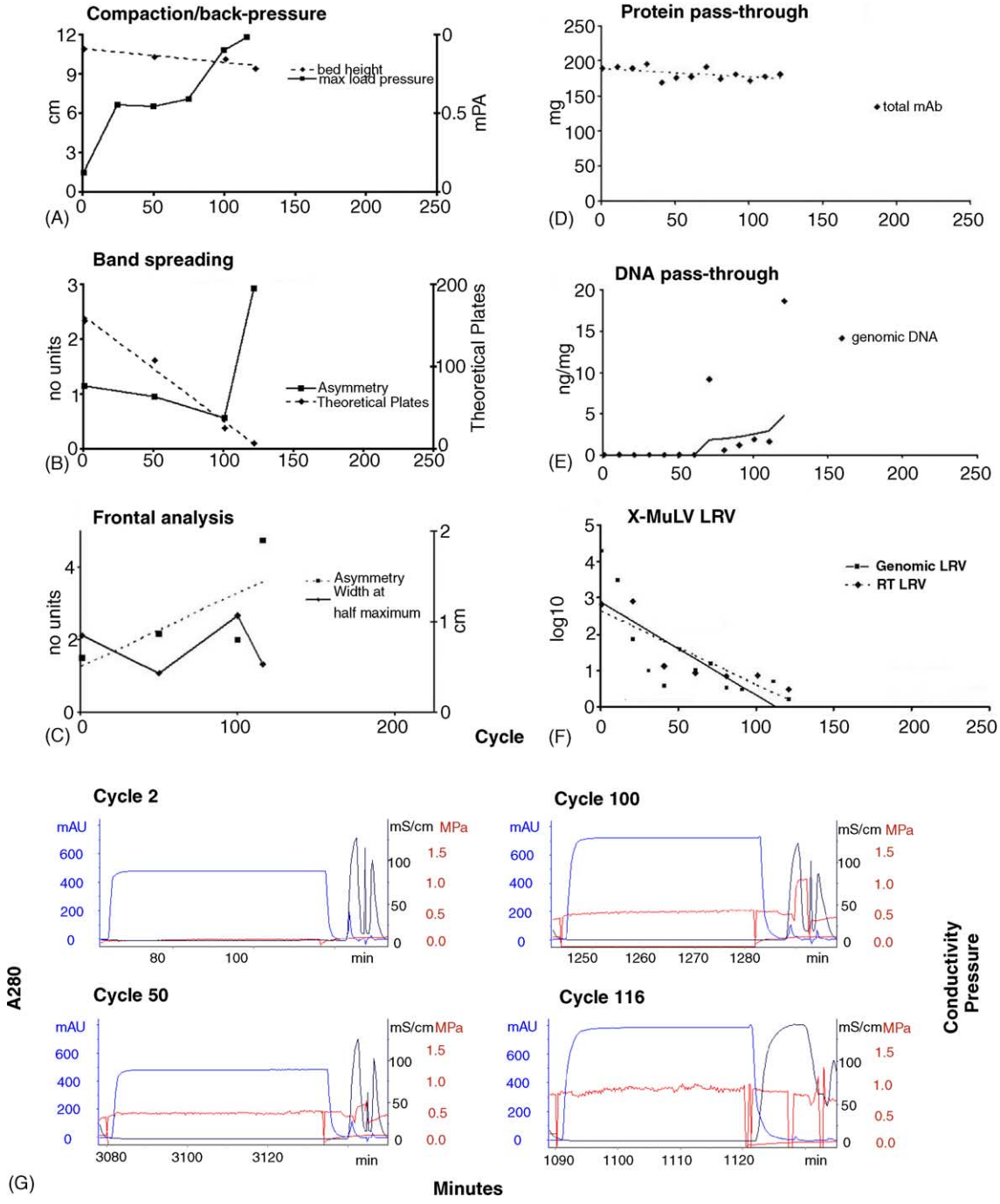

Fig. 6. Q-Sepharose fast flow column run through 122 cycles of product pass-through/regeneration/cleaning with 100 mM HCl cleaning buffer. The experiment consisted of three separate runs captured in independent run files (cycles 1–50, 51–100, 101–122). The column was not repacked. (A–G) Panel identifications and use of lines and symbols are the same as Fig. 5.

MVM (Table 1). Like X-MuLV clearance, recycled resins from all three manufacturers were equivalent to naïve resins in their capacity to clear these viruses. The Q-Sepharose fast flow and Q-Ceramic Hyper D resins were somewhat more effective at clearing MVM than Toyopearl super Q-650M.

Packing quality at the beginning and over the duration of the 200 cycles were within an acceptable range; theoretical plates of the columns ranged from 50 to 170 and showed little trend either upwards or downward over time or variation between vendors (Figs. 2B, 3B and 4B). Significant variation in only one attribute was observed among resins with different matrix chemistries; the polystyrene/ceramic-based Q-Ceramic hyper D column ran at higher operating pressure (0.3–0.7 mPA) than the other resins (Figs. 2A, 3A and 4A). However, the backpressure remained below 1 mPA over the duration of the study, did not contribute to resin compaction,

and X-MuLV LRV appeared unaffected (Fig. 3F). The shapes of the chromatograms were similar over the duration of the cycling (Fig. 2G, data not shown), demonstrating consistency of the small-scale model process over extended cycling. Similar consistency was observed in chromatograms of the other resin types (data not shown). In all three cases, complete genomic DNA clearance was also noted over 200 cycles (Figs. 2E, 3E and 4E). Protein carry-over was minimal (<0.1% of load) or undetectable in the blank cycles, while X-MuLV nucleic acid was undetectable in the blank cycles (data not shown).

3.2. Multiple cycling of Q-Sepharose without cleaning

Multiple cycling of anion-exchange resins without cleaning has been observed to lead to the loss of protein capacity because incomplete cleaning allows resin fouling, even with NaCl-based regeneration [4–6,9]. When we tested the performance of Q-Sepharose repeatedly cycled without cleaning, fouling was evidenced as a clear upward trend of backpressure leading to resin compaction (Fig. 5A and G). The backpressure terminated the experiment at cycle 82 because it eventually exceeded 1 mPA. The fouling also diminished the number of theoretical plates of the column (160–51, Fig. 5B) and had a significant impact on X-MuLV LRV by cycle 82 (Fig. 5F). A more modest impact was seen on SV40 and MVM clearance, perhaps because the resin was re-packed before the re-test, improving resin performance. Interestingly, DNA clearance remained complete over the duration of the experiment (Fig. 5E). It is important to note that repeatedly omitting resin cleaning is not common industry practice and is not generally considered to be good manufacturing practice (GMP).

3.3. Multiple cycling of Q-Sepharose cleaned with 0.1 M HCl

HCl-based cleaning solutions are known to gradually degrade sepharose-based resins, and are generally not recommended for cleaning anion-exchange resins [9,10]. Acidic cleaning is believed to hydrolyse glycosidic bonds in the agarose matrix, and has typically been manifest as accelerated leakage of total organic carbon, ligands and ligand derivatives [9,10]. Thus, this cleaning buffer can be used in forced degradation studies where resin stability performance can be monitored as they decay. When we tested the performance of HCl-cleaned Q-Sepharose, matrix disintegration was evident as a clear upward trend of backpressure (Fig. 6B). Again, backpressure terminated the experiment at cycle 122 because it exceeded 1 mPA. The acid-induced resin degradation also diminished the number of theoretical plates of the column to a great degree (155–7, Figs. 6B and 7A). Distortion and asymmetry of the acetone peak clearly indicated band spreading and uneven flow through the degraded column. A more subtle distortion of the shape of the protein load front curve was also evident at cycle 122, both upon visual examination of the curve and as an increase in the asymmetry of the first derivative of the UV absorbance curve (Figs. 6C, 6G and 7B). Close examination of the chromatograms also revealed a gradual decrease in the amount of UV absorbing material stripped from the column during regeneration (Fig. 6G).

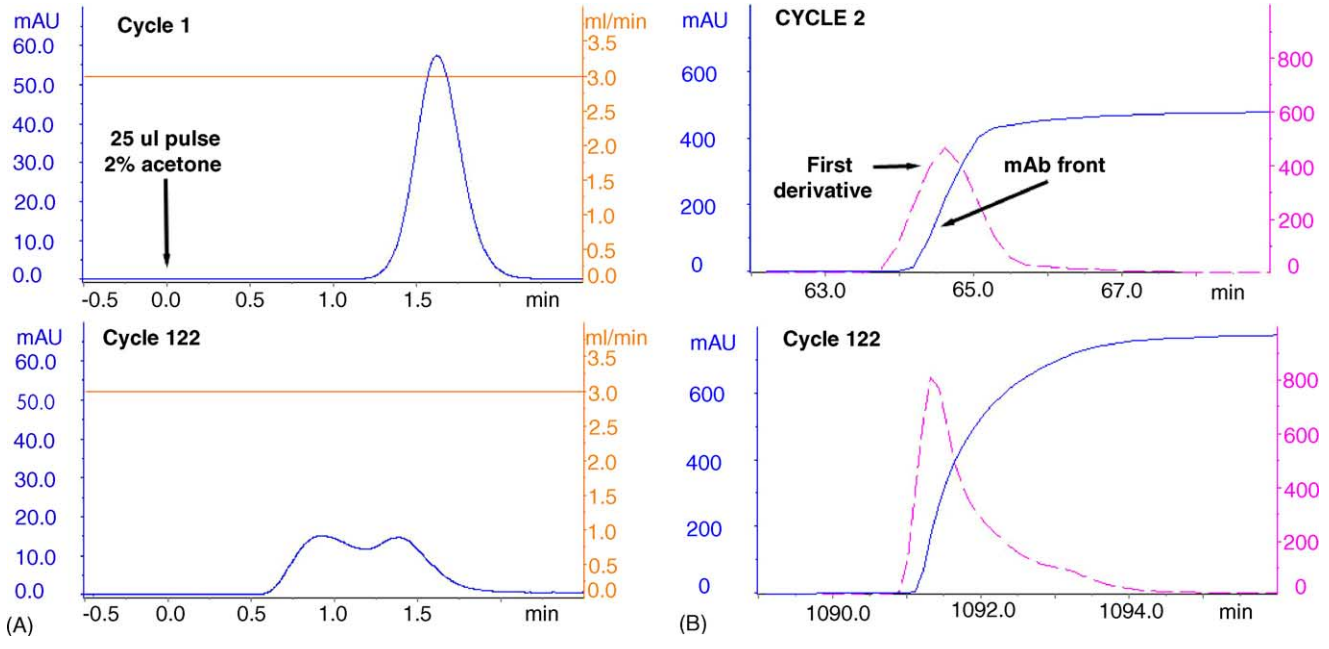

Fig. 7. Comparison of band spreading and frontal analysis at cycles 1 and 122 of the HCl cleaned QSFF resins. (A) The blue line is the A_{280} reading in mAU as the 2% acetone pulse traverses the column. The orange line is the H_2O mobile phase flow held constant at 3 ml/min. (B) The blue line is the A_{280} reading as the leading edge of the mAb load exits the column. The purple line is the first derivative of this curve.

The resin degradation had a clear negative impact on X-MuLV LRV as measured by both the X-MuLV genome Q-PCR and Q-PERT assays (Fig. 6F). The impact on SV40 and MVM clearance was more modest (~1 $\log_{10}$, Table 1), but may represent an underestimate due to sensitivity limitations of the Q-PCR assays. Also, the resin was re-packed prior to the measurement of their SV40 and MVM clearance capacity, potentially restoring some chromatographic performance.

Resin disintegration and probably ligand loss was so profound by cycle 70 that DNA (a highly negatively charged molecule predicted to bind avidly to Q-Sepharose) breakthrough was evident (Fig. 6E). DNA breakthrough is highly indicative of extensive ligand/binding site loss, arguing that the virus clearance capacity diminished due to loss of potential virus binding sites over time.

Extensive fouling in never-cleaned resins is also predicted to result in binding site loss via blockage of functional groups, similarly diminishing viral clearance. In contrast, NaOH-based cleaning buffers will strip off impurities blocking functional groups with out destroying the resin. Our observation that viral clearance is stable under this condition is consistent with the hypothesis that the number of potential binding sites will not change significantly over extended processing with NaOH-based cleaning.

Many of these performance attributes are normally monitored on a batch-by-batch basis in commercial processes to detect declining column performance. In commercial practice, media that start to fail in-process action limits or other performance targets are discarded because of regulatory considerations or the quality of the overall process becomes adversely impacted [1,2,13]. It should be noted that protein pass-through (step yield) remained constant in all five experiments, including in the cases of clear fouling or matrix/ligand disintegration (Figs. 2D, 3D, 4D, 5D and 6D). This argues that step yield is not a particularly useful parameter to predict flow-through mode anion-exchange column performance in this context. Finally, it should be noted that routine use of destructive cleaning buffers is not common industry practice, nor is it generally considered to be GMP.

4. Conclusions

We find that anion-exchange columns that are cleaned with recommended buffers (e.g. NaOH-based solutions) do not lose viral clearance capacity after extensive re-use. In contrast, anion-exchange columns that are not cleaned or are cleaned with buffers that chemically degrade the media (e.g. HCl) gradually lose viral clearance capacity. In these cases, other performance attributes that change at the same time and that are routinely monitored during commercial manufacturing include increased band spreading, decreased DNA clearance and accumulating backpressure that prevented re-use past 80–120 cycles. Thus, our data suggests that flow through mode anion-exchange columns that are cleaned with recommended cleaning buffers, and periodically monitored for theoretical plates, DNA clearance and/or backpressure need not be re-evaluated for viral clearance at the end of the validated media lifetime.

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References

[1] R. Seely, H. Wight, H. Fry, S. Rudge, G. Slaff, BioPharm 7 (1994) 41.
[2] R. O'Leary, D. Feuerhelm, D. Peers, Y. Xu, G.S. Blank, BioPharm 14 (2001) 10.
[3] Anonymous, Viral safety evaluation of biotechnology products derived from cell lines of human or animal origin, Q5A, International Conference for Harmonization of Technical Requirements for Registration of Pharmaceuticals for Human Use, Geneva, Switzerland, 1998.
[4] P. Levison, A. Hopkins, P. Hathi, J. Chromatogr. A 865 (1999) 3.
[5] P. Tice, I. Mazsaroff, N. Lin, F. Regnier, J. Chromatogr. 410 (1987) 43.
[6] M. Asplund, M. Ramberg, Downstream 28 (1998) 7.
[7] G. Hale, A. Drumm, P. Harrison, J. Phillips, J. Immunol. Methods 171 (1994) 15.
[8] K. Brorson, J. Brown, E. Hamilton, K.E. Stein, J. Chromatogr. A 989 (2003) 155.
[9] A. Adner, G. Sofer, BioPharm 7 (1994) 44.
[10] M. Andersson, M. Ramberg, B.L. Johansson, Process Biochem. 33 (1998) 47.
[11] B.L. Johansson, U. Hellberg, O. Wennberg, J. Chromatogr. 403 (1987) 85.
[12] B. Johansson, BioPharm 5 (1992) 34.
[13] Y. Xu, K. Brorson, Dev. Biol. 113 (2003) 89.
[14] K. Brorson, P.G. Swann, E. Lizzio, T. Maudru, K. Peden, K.E. Stein, Biotechnol. Prog. 17 (2001) 188.
[15] S. Curtis, K. Lee, G.S. Blank, K. Brorson, Y. Xu, Biotechnol. Bioeng. 84 (2003) 179.
[16] A. Karger, B. Bettin, H. Granzow, T. Mettenleiter, J. Virol. Methods 70 (1998) 219.
[17] J. Transfiguracion, A. Bernier, N. Arcand, P. Chahal, A. Kamen, J. Chromatogr. B, Biomed. Sci. Appl. 761 (2001) 187.
[18] N. Brument, R. Morenweiser, V. Blouin, E. Toublanc, I. Raimbaud, Y. Cherel, S. Folliot, F. Gaden, P. Boulanger, G. Kroner-Lux, P. Moullier, F. Rolling, A. Salvetti, Mol. Ther. 6 (2002) 678.
[19] A. Carlsson, J. Kuznar, M. Varga, E. Everitt, J. Virol. Methods 47 (1994) 27.
[20] D. Debelak, J. Fisher, S. Iuliano, D. Sesholtz, D.L. Sloane, E.M. Atkinson, J. Chromatogr. B, Biomed. Sci. Appl. 740 (2000) 195.
[21] B.G. Huyghe, X. Liu, S. Sutjipto, B.J. Sugarman, M.T. Horn, H.M. Shepard, C.J. Scandella, P. Shabram, Hum. Gene Ther. 6 (1995) 1403.
[22] M. Kuiper, R.M. Sanches, J.A. Walford, N.K. Slater, Biotechnol. Bioeng. 80 (2002) 445.

[23] R.S. O'Keeffe, M.D. Johnston, N.K. Slater, Biotechnol. Bioeng. 62 (1999) 537.
[24] K. Sugawara, K. Nishiyama, Y. Ishikawa, M. Abe, K. Sonoda, K. Komatsu, Y. Horikawa, K. Takeda, T. Honda, S. Kuzuhara, Y. Kino, H. Mizokami, K. Mizuno, T. Oka, K. Honda, Biologicals 30 (2002) 303.
[25] S. Zolotukhin, M. Potter, I. Zolotukhin, Y. Sakai, S. Loiler, T.J. Fraites Jr., V.A. Chiodo, T. Phillipsberg, N. Muzyczka, W.W. Hauswirth, T.R. Flotte, B.J. Byrne, R.O. Snyder, Methods 28 (2002) 158.
[26] B.L. Johansson, C. Ellstrom, J. Chromatogr. 330 (1985) 360.
[27] B.L. Johansson, L. Ahsberg, J. Chromatogr. 351 (1986) 136.
[28] A. Berggrund, I. Derevin, K. Knuuttila, J. Wardhammar, B. Johansson, Process Biochem. 29 (1994) 455.
[29] M. Andersson, M. Ramberg, B.L. Johansson, Process Biochem. 33 (1998) 47.
[30] U. Hellberg, J. Ivarsson, B. Johansson, Process Biochem. 31 (1996) 163.
[31] B.L. Johansson, J. Gustavsson, J. Chromatogr. 457 (1988) 205.
[32] Anonymous, Points to consider in the manufacture and testing of monoclonal antibody products for human use, Food and Drug Administration, US Department of Health and Human Services, Rockville, MD, 1997.
[33] T. Larson, J. Davis, H. Lam, J. Cacia, Biotechnol. Prog. 19 (2003) 485.
[34] J.C. Giddings, Dynamics of Chromatography, Dekkar, New York, 1965.
[35] E. Boschetti, L. Guerrier, P. Girot, J. Horvath, J. Chromatogr. B, Biomed. Appl. 664 (1995) 225.
[36] Y. Kato, K. Komiya, T. Iwaeda, H. Sasaki, T. Hashimoto, J. Chromatogr. 205 (1981) 185.
[37] K. Brorson, Y. Xu, P.G. Swann, E. Hamilton, M. Mustafa, C. de Wit, L.A. Norling, K.E. Stein, Biologicals 30 (2002) 15.
[38] T. Maudru, K.W. Peden, Biotechniques 25 (1998) 972.
[39] L. Shi, Q. Chen, L.A. Norling, A.S. Lau, S. Krejci, Y. Xu, Biotechnol. Bioeng. 87 (2004) 884.
[40] L. Shi, J. Ho, L.A. Norling, M. Roy, Y. Xu, Biologicals 27 (1999) 241.
[41] L. Shi, L.A. Norling, A.S. Lau, S. Krejci, A.J. Laney, Y. Xu, Biologicals 27 (1999) 253.
[42] D. Zhan, M. Roy, C. Valera, J. Cardenas, J. Vennari, J. Chen, S. Liu, Biologicals 30 (2002) 259.
[43] P. Levison, S. Badger, R. Jones, D. Toome, M. Streater, N. Pathirana, D. Wheeler, J. Chromatogr. A 702 (1995) 59.
[44] Y. Dasarathy, BioPharm 9 (1996) 41.