Effects of pH, Conductivity, Host Cell Protein, and DNA Size Distribution on DNA Clearance in Anion Exchange Chromatography Media

Melani C. Stone
Dept. of Purification Sciences, MedImmune, Gaithersburg, MD 20878

Jon Borman
Dept. of Analytical Sciences, MedImmune, Gaithersburg, MD 20878

Gisela Ferreira
Dept. of Purification Sciences, MedImmune, Gaithersburg, MD 20878

P. David Robbins
Dept. of Purification Sciences, MedImmune, Gaithersburg, MD 20878

DOI 10.1002/btpr.2556
Published online September 30, 2017 in Wiley Online Library (wileyonlinelibrary.com)

Flowthrough anion exchange chromatography is commonly used as a polishing step in downstream processing of monoclonal antibodies and other therapeutic proteins to remove process-related impurities and contaminants such as host cell DNA, host cell proteins, endotoxin, and viruses. DNA with a wide range of molecular weight distributions derived from Chinese Hamster Ovary cells was used to advance the understanding of DNA binding behavior in selected anion exchange media using the resin (Toyopearl SuperQ-650M) and membranes (Mustang® Q and Sartobind® Q) through DNA spiking studies. The impacts of the process parameters pH (6–8), conductivity (2–15 mS/cm), and the potential binding competition between host cell proteins and host cell DNA were studied. Studies were conducted at the least and most favorable experimental conditions for DNA binding based on the anticipated electrostatic interactions between the host cell DNA and the resin ligand. The resin showed 50% higher DNA binding capacity compared to the membrane media. Spiking host cell proteins in the load material showed no impact on the DNA clearance capability of the anion exchange media. DNA size distributions were characterized based on a "size exclusion qPCR assay." Results showed preferential binding of larger DNA fragments (>409 base pairs). © 2017 The Authors Biotechnology Progress published by Wiley Periodicals, Inc. on behalf of American Institute of Chemical Engineers *Biotechnol. Prog.*, 34:141–149, 2018
Keywords: DNA clearance, anion exchange chromatography

Introduction

Host cell DNA (DNA) is a common process-related impurity derived from host cells used in the manufacturing of biologics, such as monoclonal antibodies (mAbs). Host cell DNA poses a concern related with the immunogenicity and safety (oncogenicity, infectivity, and immunomodulatory effects).[1–3]

The World Health Organization (WHO) recommends an acceptable limit of 10 ng DNA/dose based on their overall DNA safety risk assessment, which is widely used as a standard in the biotech industry.[4] A study performed by the Center for Biologics Evaluation and Research showed that DNA fragments smaller than 200 base pairs (bp) reduce the probability of intact oncogenes and other functional sequences that may be introduced into drug product for possible integration events.[4]

Host cell DNA, along with other product- and process-related impurities, is removed in downstream purification processes for biopharmaceuticals. These processes typically comprised of a capture step followed by two or more polishing steps, which usually includes an anion exchange (AEX) chromatography step. DNA clearance is attained over multiple process steps to acceptable levels in the Drug Substance, according to the WHO recommendation.

Significant DNA clearance is usually achieved over the capture step, posing an analytical challenge to accurately understand the capability of the AEX chromatography to remove host cell DNA, because this step is generally used as a polishing step and host cell DNA levels in the load and products are close to the level of quantification of the analytical assay. Thus, spiking studies were used to demonstrate and understand

Current address of Melani C. Stone: Department of Biologics Process Development and Commercialization, Merck Manufacturing Division, Merck & Co., Inc., West Point, PA 19486, USA

Correspondence concerning this article should be addressed to P. D. Robbins at RobbinsD@medImmune.com.

the clearance capability of host cell DNA using selected membranes and resin AEX chromatography media.

mAbs of the subclass IgG1 typically have basic pIs (around 7–9).[5,6] An AEX chromatography step is often run in flowthrough mode at neutral to slightly basic pH as a polishing step for removal of impurities such as viruses and DNA which are expected to bind to the media while the product is collected in the nonbound fraction.

Results demonstrate the impacts of factors such as pH, conductivity (controlled by salt concentration), the level of host cell protein (HCP), and the size distribution of DNA molecules on process robustness and capability for DNA clearance in AEX, when operated in flowthrough. All model AEX chromatography media (Toyopearl SuperQ-650M resin and membranes Mustang® Q and Sartobind® Q) were based on quaternary ammonium to represent the common use of this chemistry in biopharmaceuticals downstream processing. The operational range for conductivity and pH was based on conditions for which effective viral clearance was measured (assumed to be over 4 $\log_{10}$ for the Xenotropic Murine Leukemia Virus), based on in-house information. Several published studies have demonstrated robust viral clearance on various AEX chromatography media under comparable pH and conductivity ranges studied in this article.[7,8]

Overall, the data presented in this study expand the understanding of DNA clearance over AEX chromatography media over a broad set of operating conditions, when operated as a flowthrough.

Materials and Methods

Preparations of DNA stock solutions for the spiking experiments

The host cell DNA (DNA) used to spike the AEX chromatography load materials in the experiments described in this article was extracted from cell paste of a Chinese Hamster Ovary (CHO) cell line used to manufacture the model mAb used in this study. DNA stock solutions were extracted from DNA produced from cell paste. The cell paste was resuspended in 1% sodium dodecyl sulfate (SDS), 2% polysorbate 20, 10 mM ethylenediaminetetraacetic acid (EDTA), and 20 μg/mL Proteinase K and then incubated in a rotator for 2 h at 56°C. An equal amount of phenol, chloroform, and isoamyl alcohol (25:24:1) was then added and mixed by inversion. This solution was centrifuged to remove the aqueous layer and then combined with an equal volume of 100% isopropanol. Another centrifugation was performed to obtain a pellet of nucleic acids containing riboxynucleic acid (RNA) and DNA. These pellets were suspended in 10 mM Tris-HCl, 1 mM EDTA, pH 8.0 buffer where the RNA was removed by adding 0.5 μg/mL RNAse A into the solution followed by incubation for 1 h at 37°C (except for the host cell DNA material that was directly purified from the cell-free culture supernatant). An equal amount of phenol, chloroform, and isoamyl alcohol (25:24:1) was then added and mixed by inversion. The solution was centrifuged to remove the aqueous layer and then combined with an equal volume of 100% isopropanol. The resulting mixture was centrifuged to obtain a pellet of unsheared DNA, which was then solubilized in 10 mM Tris-HCl, 1 mM EDTA, pH 8.0 buffer. All reagents were from Life Technologies, MD, with exception of polysorbate 20 and Proteinase K which were obtained from Sigma Aldrich, MO and Promega, WI, respectively.

In order to characterize the DNA size distribution in the cell-free culture supernatant and in the load material for all the experiments, a portion of the DNA obtained from the cell paste was sonicated until the size distribution profile was comparable to that of the DNA from a typical sample of the cell-free culture supernatant of the antibody process using a Q500 sonicator (Qsonica, CT) at 50% amplitude for 10 s. The characterization of DNA size distribution was performed using the Bioanalyzer 2100 (Agilent, CA). For the spiking studies, a mixture containing equal volumes of sheared and unsheared DNA was used to provide a wide range in molecular size.

Monoclonal antibody, reagents, and chromatography media

The load material for all experiments used a sample of an IgG_1 mAb of concentration 7.9 mg/mL with pI of 8.9–9.1 in approximately 76 mM phosphate buffer, 25 mM sodium chloride containing 1.43 pg DNA/mg mAb and over 95% monomer by high performance size exclusion chromatography. The HCP stock solution was obtained from null CHO cells generated from a cell line that was grown under similar conditions to the mAb producing cells. All reagents for buffers were purchased from J.T. Baker, PA.

The AEX chromatography media studied were the resin Toyopearl SuperQ-650M, referred to as SuperQ (Tosoh Bioscience LLC, PA), and the membranes Mustang® Q XT5 (Pall Corporation, NY) and Sartobind® Q Pico (Sartorius Stedim Biotech, NY), referred to as Mustang Q and Sartobind Q, respectively. The SuperQ column was packed at a compression of 1.2 to a 19.6 cm bed height × 0.66 cm bed diameter (6.7 mL column volume). The Mustang Q XT5 used a capsule with membrane volume of 5 mL and Sartobind Q Pico used a capsule with a membrane volume of 0.08 mL.

Equipment

Chromatography runs were conducted on an AKTA Explorer (GE Healthcare, Uppsala, Sweden). Runs using membranes were performed using a peristaltic pump Masterflex L/S (Cole-Parmer, IL).

Chromatography methods

Spiking studies were performed in two parts. For the study in Part 1, the AEX chromatography load was prepared by adjusting the purified process intermediate to pH 6 and 15 mS/cm with 1 M acetic acid and 4 M sodium chloride solutions before the addition of DNA stock solution. For the study Part 2, DNA and HCP stock solution were added into 12 mM sodium phosphate pH 8.0 buffer without any mAb. No significant change was observed on pH and conductivity after host cell DNA and/or HCP spike so no further adjustments were needed. Load materials were prepared fresh prior to each run.

All experiments were performed under isocratic chromatography conditions. Membrane experiments were operated at flow rates of 10 membrane volumes/minutes (MV/min) for both parts of the study. SuperQ experiments were conducted at a linear velocity of 500 cm/h for study Part 1 and at 300 cm/h for study Part 2.

Resin and membranes were pre-equilibrated with 312 mM sodium phosphate pH 7.5 buffer and then equilibrated with

Table 1. Summary of Experimental Conditions Bracketing the Least and Most Favorable Binding Conditions of Host Cell DNA to the Anion Exchange Media Within the Studied Range

Process Parameter	Part 1: Least Favorable Conditions for DNA Binding	Part 2: Most Favorable Conditions for DNA Binding
Pre-equilibration buffer	312 mM sodium phosphate pH 7.5	
Buffer composition	70 mM sodium phosphate, 90 mM sodium chloride, pH 6.0	12 mM sodium phosphate, pH 8.0
Conductivity (mS/cm)	15	2
Flow rate (min)	500 cm/h (resin) 10 MV/min (membranes)	300 cm/h (resin) 10 MV/min (membranes)

70 mM sodium phosphate, 90 mM sodium chloride, pH 6.0 (study Part 1), or 12 mM sodium phosphate pH 8.0 buffers (study Part 2) to match the load pH and conductivity. At the end of the load, the equilibration buffer was used to recover the product. Product fractions were collected and analyzed for host cell DNA and HCP content.

Protein load as measured by the antibody concentration was targeted to be 200 mg/mL for the resin and 20,000 mg/membrane mL-equivalent for the Sartobind Q and Mustang Q.

Experimental conditions are summarized in Table 1.

Analytical techniques

Antibody concentration was determined spectrophotometrically by UV absorbance (wavelength of 280 nm).

HCPs concentration was measured by a sandwich immunoassay using sheep anti-CHO HCP (MedImmune, Gaithersburg, MD) as a capture and detection antibody. HCP level is calculated based on a standard curve using HCP prepared from null CHO HCP reagents. HCP was spiked at a ratio of 1.1–1.4 ng/ng of DNA.

Host cell DNA content was measured by a quantitative PCR (qPCR) assay. This qPCR method used forward and reverse 64 bp primers targeting highly repetitive Short Interspersed Nuclear Element (SINE) regions in the CHO genome. Prior to DNA analysis, samples were combined with chaotropic salts and precipitated with isopropanol to isolate nucleic acids. CHO DNA levels were calculated based on a standard curve using purified CHO genomic DNA.

Size distribution of DNA: two analytical methods were used for size distribution determination based on gel electrophoresis (1) and a PCR based assay (2).

1. *Gel electrophoresis*: The method used the Bioanalyzer 2100 with DNA high sensitivity chip to provide a quantitative measurement for DNA amounts in the order of $\geq$500 pg.
2. *Size exclusion qPCR*: This method allows for qualitative measurement of DNA size distribution for a sample with residual quantity of DNA sample ($\leq$500 pg). In this method, a series of PCR reactions targeting overlapping targets of different sized regions of the CHO genome were performed. The PCR targets the Long Interspersed Nuclear Element (LINE) sequences interspersed throughout the CHO genome. A shared forward primer FWD: TCCATCCCAATCAAGTAGGC is utilized with two reverse primers REV53: TCCCTGGGATGAAGCCTACT, and REV409: ATTCCCTTGATCGCCTTTTG to generate 53 and 409 bp products respectively. The principle behind this technique is that DNA fragments of a size smaller than the target size cannot be amplified. A population of DNA fragments all under 409 bp would generate no PCR product using a primer set targeting a 409 base pair region in the genome. For this study, the size information is presented as an amplification ratio (AR), which is expressed as a percentage of PCR amplification of a 409 bp target relative to that of a 53 bp target. In relative terms, the % amplification ratio (% AR) for a DNA population consisting of nonfragmented DNA would be 100%. Likewise, a homogeneous population of DNA shorter than 409 bp would have a 0% AR. The principle of the method is schematically exemplified in Figure 1.

Results and Discussion

Experimental design selection

Several published studies have demonstrated the effectiveness of AEX chromatography media for DNA removal,[9–12] either as resin or as a membrane format. These studies were conducted using DNA that was not host cell specific or not representative for size distribution of the DNA generated in the cell culture process. In a specific study, Butler et al.[13] conducted DNA spiking studies using both commercial DNA and host cell specific DNA on an AEX chromatography resin at conditions that favor DNA binding (pH 8 and low conductivity). The DNA stock solution was sheared to have representative DNA size distribution to that typically seen in the process. However, their study was limited to a single favorable condition for DNA binding. This study determined the robustness of AEX chromatography for DNA clearance under less favorable operating conditions such as lower pH and higher conductivity values, or in presence of competing binding HCPs.

The mode of separation of an AEX chromatography resin is based on electrostatic interactions between negative charges (such as viruses, HCP, and DNA) and the positively charged ligand. Consequently, load pH and conductivity were identified as parameters that may have impact on the clearance of virus, DNA, and HCP. The pH and conductivity ranges were selected based on the region where viral clearance capability for Xenotropic Murine Leukemia Virus was greater than 4 $\log_{10}$ reduction value (LRV), as indicated by the dotted line in Figure 2. A full factorial design would determine the interactions between the pH and conductivity at all four corners of the regions, which would result in four runs in addition to one center point condition. The study was simplified by using a bracketing strategy, aiming at the two experimental conditions to cover the least and most favoring conditions for DNA binding, based on minimal and maximum interactions with the resin, as determined by the pH and conductivity of the load material.

The spiking study was divided into two parts: Part 1 was the least favorable conditions for DNA binding (lowest pH and highest conductivity) within the experimental range to determine the minimum DNA clearance capability of the AEX chromatography step. DNA binding in AEX matrices

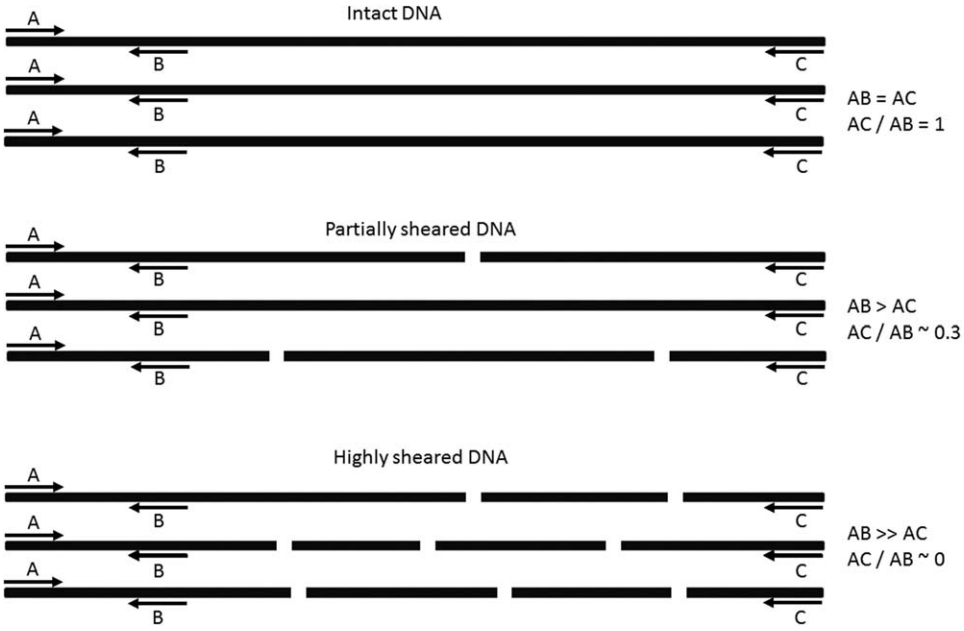

Figure 1. Illustration of the principle of "size exclusion qPCR method" for qualitative measurement of DNA size distribution. Increasing degree of DNA shear shifts the distribution to smaller fragments, decreasing the AC/AB ratio.

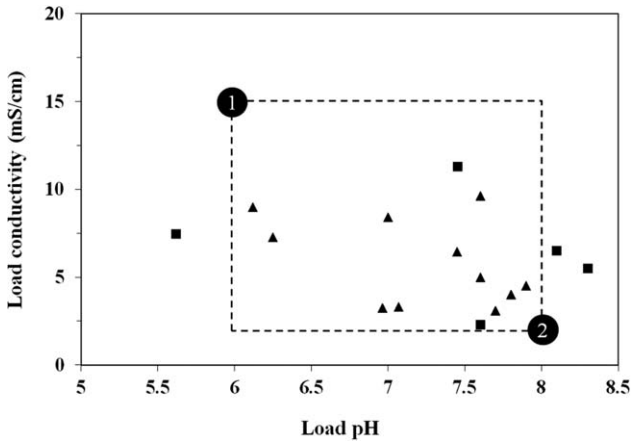

Figure 2. Historical data for Xenotropic Murine Leukemia Virus clearance for the SuperQ resin. Solid triangles (▲) indicate LRV > 6, while solid squares (■) indicate measurements between 4 and 6 LRV. Dotted lines represent the risk assessment and process characterization ranges for DNA spiking study. Point 1 corresponds to the least favorable conditions for DNA binding—experimental conditions for Part 1 study. Point 2 corresponds to the most favorable conditions for competitive bindings of DNA and HCP—experimental conditions for Part 2 study.

has been shown to be favored at high pH and low conductivity conditions.[10,13,14] Combination of low pH and high conductivity would be expected to reduce the electrostatic interactions between DNA and the ligand, by potentially reducing the effective charge of DNA. SuperQ is typically run at a linear velocity ranging from 200 to 500 cm/h. In this study, the flow rate for SuperQ was set at the highest value to minimize the residence time and, theoretically, increase the mass transfer resistance for DNA transport into the pores. The membranes were run at target flow rate as dynamic binding capacity is typically independent of flow rate due to the convective flow nature of a membrane.[15] Part 2 of the study used the most favorable conditions for DNA binding (highest pH and lowest conductivity). At these conditions, HCPs will tend to bind more strongly, thus increasing the chance for competitive binding between HCPs with DNA. SuperQ flow rate was set at the linear velocity of 300 cm/h. The mAb load concentration was smaller for the chromatography resin than that used for membranes, based on the maximum mAbs load values used in-house for these two AEX media.

DNA stock characterization

Results from DNA characterization are shown in Figure 3. Host cell DNA from the cell-free culture supernatant has a typical size distribution of 700–10,000 bp with peak maximum at around 4,000 bp (Figure 3a). The host cell DNA extracted from the cell paste has size distributions from 2,000 bp to greater than 10,000 bp fragment size (Figure 3b). Figure 3c shows the size distribution profile of host cell DNA extracted from the cell paste which was sheared to better match the typical profile of host cell DNA from the cell-free culture supernatant. In this study, the host cell DNA spiked into the AEX chromatography load was prepared by combining equal volumes of sheared and unsheared DNA stock solution. This combination resulted in DNA with wider size distribution than typically seen in the process to cover a broader range of DNA fragment size. The size distribution profile of the DNA spike solution is shown in Figure 3d. The use of a wider span of host cell DNA sizes than that typically found in the cell-free culture supernatant intended to better understand the impact of size on DNA clearance by the AEX chromatography media and cover wider distributions in the load material.

DNA spiking studies

The DNA binding capacity values measured in this study were lower compared to published spiking studies on AEX chromatography membranes using commercial herring sperm DNA (hs-DNA).[11,12] The differences could be attributed to

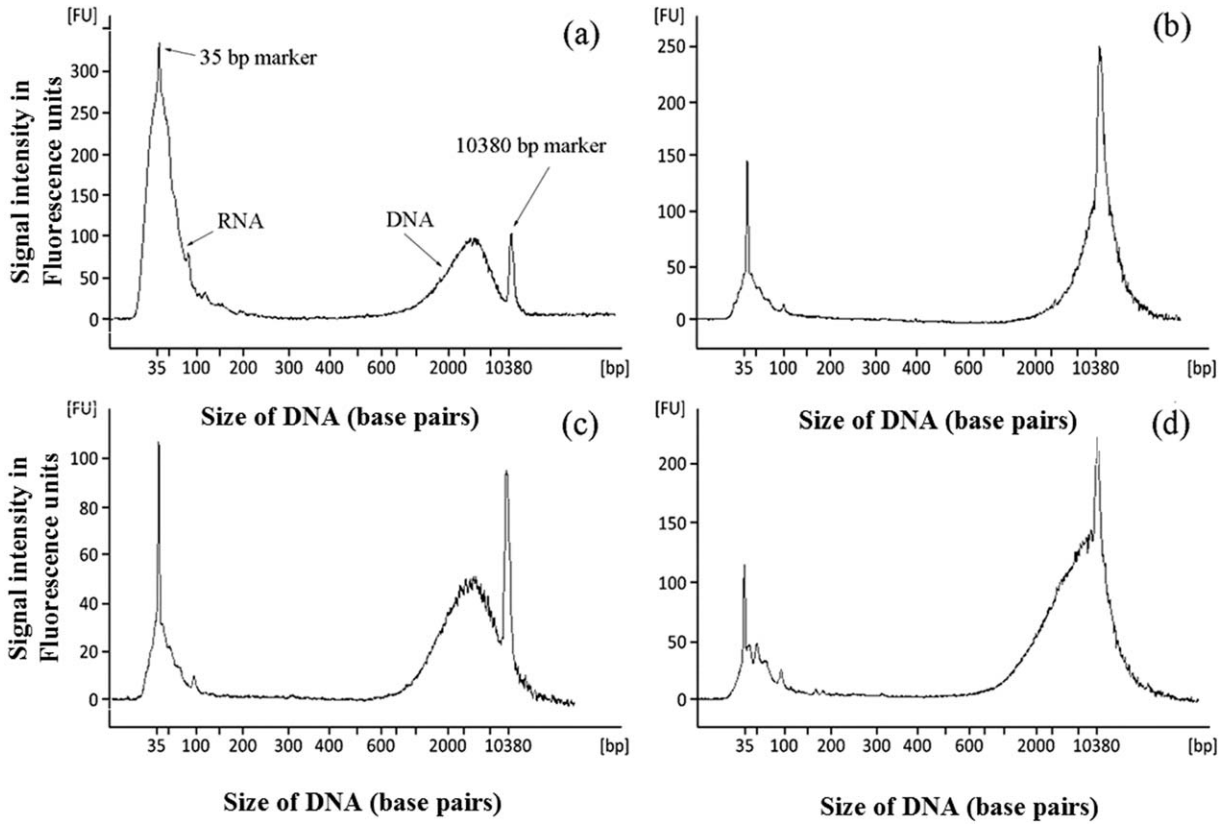

Figure 3. DNA size distribution profile from gel electrophoresis analysis of (a) typical nucleic acid size distribution from a cell-free culture supernatant, (b) unsheared host cell DNA extracted from cell paste, (c) sheared DNA extracted from the cell paste, and (d) DNA spike solution containing 1:1 mixture of sheared and unsheared starting DNA from cell paste.

several factors including operational conditions and the different types of DNA used for the spiking studies. For example, DNA binding to AEX at a higher pH can be expected to result in a higher binding capacity. The larger and broader size ranges of DNA fragments used in our study may "cover" more binding sites, thus reducing the "available" binding capacity compared to studies with smaller DNA fragments such as hs-DNA. However, the LRVs obtained in this study are relatively higher compared to the published studies because the qPCR assay used in this study was very sensitive to DNA content in the load and, more importantly, the product. The DNA used in this study was obtained through purification from the host cell paste. (Preparation from the cell-free culture supernatant was not practical due to the large volume required. Host cell paste was used because of its significantly higher DNA content). The DNA was then sheared to a representative size distribution of the cell-free culture supernatant.

DNA spiking study: Part 1 (pH 6, conductivity of 15 mS/cm)

Table 2 lists the experimental conditions of DNA spiking studies (Part 1) on three AEX chromatography media. For each AEX media, three runs were conducted with varying DNA concentration in the AEX chromatography load. The target runs were performed without any DNA spiking to provide a baseline for step mAb yield and chromatograms profiles. No impact on step yield and chromatograms were observed with DNA spiking (results not shown). The low spike (LS) and high spike (HS) runs had DNA concentrations of 95 ± 10 and 320 ± 30 ng DNA/mg mAb, respectively. For these runs, the amounts of mAb load were fixed to 211 ± 12 mg/mL of column volume and 21 ± 1 g/mL of membrane volume for the resin and membranes, respectively.

The LRV for DNA clearance was calculated according to

$$\log_{10} \text{ reduction value (LRV)} = \log_{10}\left(\frac{C_{\text{DNA,load}} \times V_{\text{load}}}{C_{\text{DNA,product}} \times V_{\text{product}}}\right) \quad (1)$$

where C is the concentration and V is the volume of the feed load or product collected, as noted.

DNA breakthrough was not observed for the SuperQ runs for either the LS or the HS conditions (Table 2). Therefore, an additional run with DNA spike of 12,000 ng DNA/mg of mAb was conducted to see if DNA breakthrough at higher DNA load would be observed. No breakthrough was seen at this very high spike level either. Results show that SuperQ resin provides $\geq$6.9 LRV of DNA clearance for a load challenge up to 2.3 mg of DNA/mL column volume. This binding capacity exceeded that of both membranes for the same load pH and conductivity.

Figures 4 and 5 show the DNA breakthrough curves for DNA load challenge up to 7 mg DNA/mL of membrane volume for Sartobind Q and Mustang Q, respectively. For Sartobind Q, DNA started to breakthrough at approximately 0.8 mg DNA/mL membrane volume for both low and high DNA spike runs. Both runs had similar DNA profiles and overlaid when plotted against the amount of DNA loaded. This result suggests that the binding capacity of DNA is independent of the DNA load concentration for the range

Table 2. Experimental Details for DNA Spiking Study at pH 6.0 and 15 mS/cm

Matrix	Run	IgG Load (mg/mL CV or MV)	DNA Concentration in Load (ng/mg mAb)	DNA Load (μg/mL CV or MV)
SuperQ	Target	220	1.43×10^{-3}	3.20×10^{-4}
	Low spike (LS) DNA ratio	216	$9.30 \times 10^{+1}$	$2.01 \times 10^{+1}$
	High spike (HS) DNA ratio	216	$2.91 \times 10^{+2}$	$6.27 \times 10^{+1}$
	Very high spike DNA ratio	194	$1.20 \times 10^{+4}$	$2.33 \times 10^{+3}$
Sartobind Q	Target	21,300	1.43×10^{-3}	3.11×10^{-2}
	Low spike (LS) DNA ratio	21,700	$8.80 \times 10^{+1}$	$1.90 \times 10^{+3}$
	High spike (HS) DNA ratio	22,000	$3.20 \times 10^{+2}$	$7.05 \times 10^{+3}$
Mustang Q	Target	20,600	1.43×10^{-3}	3.10×10^{-2}
	Low spike (LS) DNA ratio	20,700	$1.04 \times 10^{+2}$	$2.27 \times 10^{+3}$
	High spike (HS) DNA ratio	20,800	$3.51 \times 10^{+2}$	$7.67 \times 10^{+3}$

CV = column volume; MV = membrane volume.

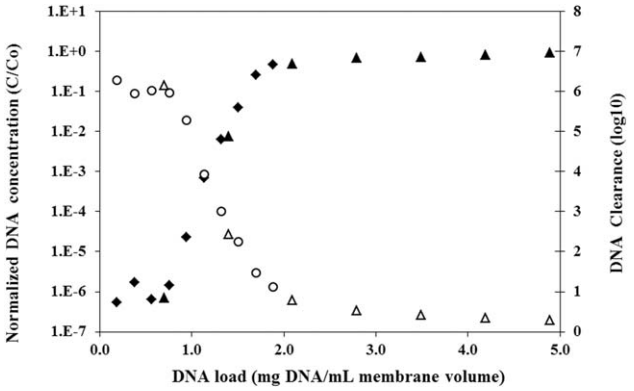

Figure 4. DNA breakthrough and DNA clearance as $\log_{10}$ reduction value for Sartobind Q at pH 6.0 and 15 mS/cm conductivity. Solid diamonds (◆) and solid triangles (▲) indicate DNA concentrations (*C*) in the flowthrough fractions relative to initial DNA concentration in the load (C_0) for low and high DNA spike ratio, respectively. Open circles (○) and open triangles (Δ) indicate log DNA clearance for low spike and high spike ratio, respectively.

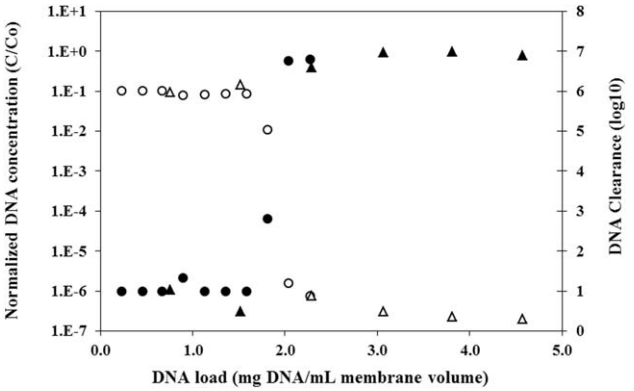

Figure 5. DNA breakthrough and DNA clearance as $\log_{10}$ reduction value for Mustang Q at pH 6.0 and 15 mS/cm conductivity. Solid circles (●) and solid triangles (▲) indicate DNA concentrations in flowthrough fractions (*C*) relative to initial DNA concentration in the load (C_0) for low and high DNA spike ratio, respectively. Open circles (○) and open triangles (Δ) indicate log DNA clearance for low spike and high spike ratio, respectively.

studied. Data suggest a clearance capability of approximately 6 LRV, up to 1 mg DNA loaded/mL of membrane.

Similar DNA profiles were also seen in Mustang Q, although the breakthrough was observed at higher DNA load (around 1.5 mg DNA/mL membrane volume). This difference in DNA binding capacity could be attributed to larger pore size of the Sartobind Q membrane which translates to less available surface area for binding.[9] LRV greater than 5 can be obtained if DNA load is limited to less than 0.8 and 1.5 mg/mL, respectively, for Sartobind Q and Mustang Q.

Table 3 summarizes the DNA clearance capability for the AEX chromatography media evaluated. At the least favorable conditions for DNA binding, SuperQ resin resulted in the highest binding capacity for DNA and provided a higher LRV compared to both membranes. Assuming 100 ng DNA/mg mAb in the cell-free culture supernatant and a maximum mAb load onto the AEX chromatography of 200 mg/mL resin, the maximum DNA load is 20 μg DNA/mL of column. The binding capacity of DNA is greater than 2.3 mg DNA/mL of column, which provides a safety factor for the SuperQ resin of more than 6.9 LRV of DNA. This analysis gives confidence on the AEX chromatography capability to clear DNA, even at the least favorable conditions for DNA binding. As a polishing step, the AEX chromatography is preceded by a capture step that by itself can typically provide a minimum of 1–3 LRV, providing a redundant step for DNA removal.

Table 3. DNA Clearance Capability at pH 6.0 and 15 mS/cm Conductivity

Media	Capacity Before DNA Breakthrough (mg DNA/mL CV or MV)	DNA Clearance ($\log_{10}$)
SuperQ	>2.3	>6.9
Mustang Q	1.5	6.0
Sartobind Q	0.8	5.9

CV = column volume; MV = membrane volume.

DNA spiking study: Part 2 (pH 8, conductivity of 2mS/cm)

A higher LRV is anticipated when running conditions at higher pH and lower conductivity through enhanced electrostatic attraction and lower charge competition with buffer species. In Part 2 study, while the pH and conductivity are the most favorable for DNA binding, these conditions could also be favorable for HCP binding. These conditions can result in binding competition between HCP and DNA and theoretically compromise DNA clearance.

The impact of HCP on DNA clearance was determined by spiking both HCP and DNA into a phosphate based buffer and running the material through SuperQ and Mustang Q. The membrane Sartobind Q was not pursued because it showed a lower binding capacity than Mustang Q. Antibody

Table 4. Summary of Part 2 DNA Spiking Study at 2 mS/cm Conductivity and pH 8.0

	SuperQ		Mustang Q	
	Without HCP Spike	With HCP Spike	Without HCP Spike	With HCP Spike
DNA load (mg/mL CV or MV)	0.58	0.60	11.9	12.5
HCP load (mg/mL CV or MV)	N/A	0.84	N/A	13.9
DNA binding capacity at breakthrough (mg/mL CV or MV)	No breakthrough	No breakthrough	4.8	4.2
DNA clearance at breakthrough ($\log_{10}$)	$\geq$8.0	$\geq$8.0	7.7	8.0
HCP clearance at breakthrough ($\log_{10}$)	N/A	$\geq$2.4	N/A	0.5

was not included in the load material because it was visibly precipitating upon HCP spiking. This effect is not observed during routine manufacturing, likely related with the much lower HCP content in a typical AEX chromatography load material relative to the spiking solution and the potential electrostatic interactions between the negatively charged HCP (which tends to be acidic[16]) and the positively charged antibody. Another reason that may explain the absence of precipitation in routine purification processes is related with the much wider HCP distribution of the HCP spiking solution as this population was not partially minimized in a prior affinity chromatography step, as it is typically implemented in an actual purification process. Another possible factor that may explain the precipitation seen in the HCP spiking study that is not observed under normal process conditions could be that the HCP spiking material had not been passed through other purification steps, and might therefore contain less soluble species not normally present at this stage of the process. It is possible that specific HCPs that would otherwise have been minimized in affinity chromatography, for example, may now be present at a concentration sufficient to induce visible precipitation. However, the conclusions of the study are not expected to be impacted by the absence of mAbs product. The antibody passes through the AEX column or membrane unbound, and therefore the presence of antibody in the load material is expected to have negligible influence over the interactions of the DNA and HCP with the anion exchanger. A spiking study with DNA only was conducted as a control run. The study results are summarized in Table 4.

For SuperQ, no impact on DNA clearance was observed when the load material was spiked with approximately 1–1 mass ratio of HCP and DNA. DNA LRV $\geq$ 8 was obtained for both DNA spiking with and without HCP spike at DNA load of 0.6 mg of DNA/mL column volume. No DNA breakthrough was observed in both runs. HCP LRV was $\geq$2.4, which was noticeably lower than DNA, indicating that DNA is preferably bound to the resin. It is likely that some subset of HCP populations such as basic proteins were not bound to the column.

A similar result was obtained for Mustang Q where presence of HCP spike has no impact on the DNA clearance. DNA broke through at a DNA load of 4.8 mg/mL (DNA spike only) and 4.2 mg/mL (DNA and HCP spikes). Similarly to SuperQ, Mustang Q also showed a stronger preference for DNA binding (8 LRV) over HCP (0.5 LRV), and higher DNA binding capacity relative to Part 1 study, consistent with the anticipated favorable DNA binding conditions.

Determination of preferential binding of DNA based on size

The size distribution of the load and selected samples of the flowthrough fractions from DNA spiking study Part 1 were analyzed by size exclusion qPCR to identify any trend of DNA binding on the AEX chromatography matrices. As described above, the size distribution of the DNA in the load to the AEX chromatography runs was comparable to Figure 3d. Upon analysis, it resulted in a 73% AR, indicating that most DNA fragments present in the load were longer than 409 bp, relative to all DNA in the solution (>53 bp), consistent with the size distribution profile obtained by gel electrophoresis.

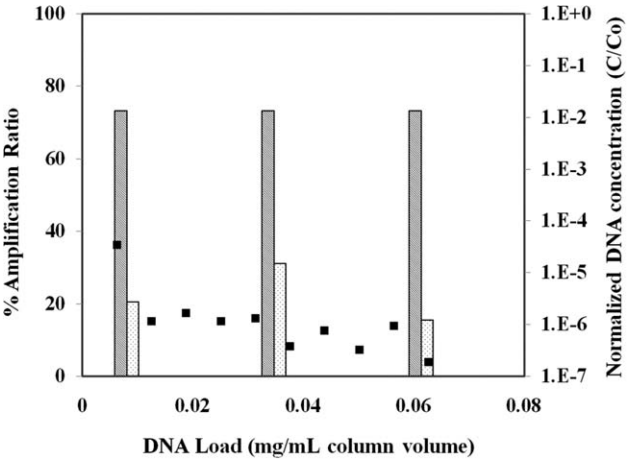

Figure 6. DNA characterizations by "size exclusion qPCR" for selected SuperQ (high spike ratio run). Solid squares (■) correspond to the *y*-axis for DNA concentration in flowthrough fraction (*C*) normalized to the initial load DNA concentration (C_0). Dotted and solid bars indicate the % of amplification ratio for selected flowthrough and load sample, respectively.

The analysis of flowthrough samples for SuperQ was performed by size exclusion qPCR only because the fractions contained a residual level of DNA < 500 pg, are not detectable by gel electrophoresis. The samples showed a 20–30% AR, indicating considerably smaller DNA size distribution compared to the load material (73% AR), as shown in Figure 6. This result suggested preferential binding for larger DNA fragments, which further assures DNA control because smaller DNA fragments have a greater safety margin.[4]

Figure 7a shows the size exclusion qPCR results for Sartobind Q fractions. Since the DNA profiles from both LS and HS coincide, the combined data points from the two runs were treated as a single profile for the size distribution characterization. The % AR increased gradually as DNA broke through and reached a plateau at 100% DNA breakthrough. The first fraction (corresponding to a point prior to DNA breakthrough) contained residual DNA level with a low 18% AR, consistent with the results obtained with SuperQ. When DNA started to breakthrough, the % AR increased to 30% and then approximately 40% at 100% DNA breakthrough.

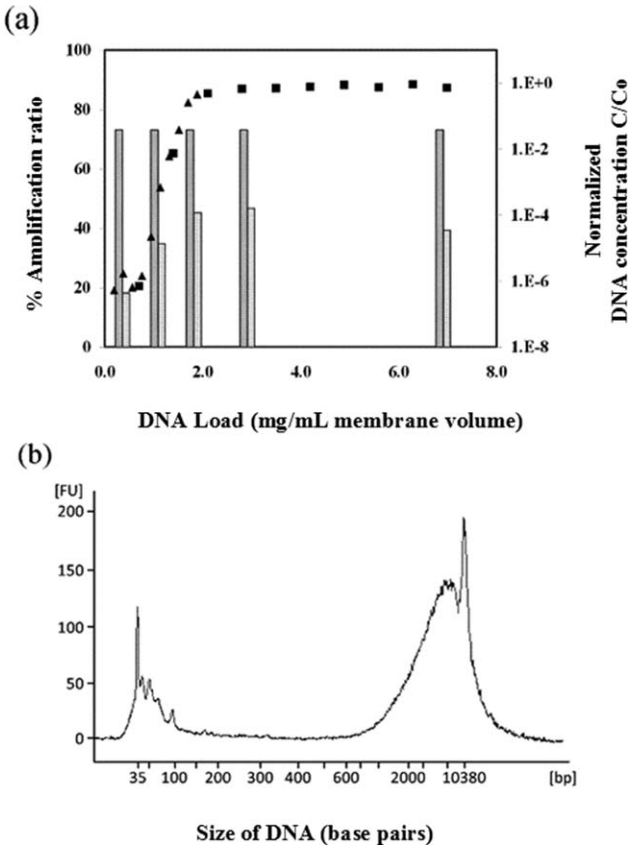

Figure 7. DNA size characterizations (a) by "size exclusion qPCR" for Sartobind Q. Solid squares and triangles (■ and ▲) correspond to the DNA concentration in the flowthrough fraction for the high and low DNA spike (C) normalized to the initial load DNA concentration (C_0). Dotted and solid bars indicate the amplification ratio for selected samples. (b) DNA size distribution profile of a fraction with an amplification ratio of 40%.

This result suggests that the host cell DNA may be partially fragmented upon running through the chromatography bed, or there may be partial displacement of lower molecular weight fragments by larger DNA molecule sizes, as the media is saturates and the DNA is breaking through. Figure 7b shows the size distribution profile of a fraction with 40% AR. The profile shows a resolved peak maximum at approximately 8,000 bp compared to the load material (Figure 3d), indicating that the unbound DNA has smaller size distributions compared to the DNA profiles of the load material. A similar study performed with Mustang Q yielded comparable results.

Conclusion

We have evaluated the impact of process parameters (pH and conductivity), DNA size distribution and competition by HCP on DNA clearance capability and binding behavior on AEX chromatography media in the flowthrough mode. A LRV $\geq$ 5.9 and 8 were obtained for all AEX chromatography media evaluated under nonfavorable binding conditions for DNA (pH 6.0 and 15 mS/cm) and favorable DNA binding conditions (pH 8.0 and 2 mS/cm), respectively. The resin showed higher binding capacity for DNA, likely related with the larger available area for binding of a resin, compared with the same volume for a membrane. Membranes were capable to bind a minimum of 0.8 mg DNA/mL membrane volume. In presence of HCP, DNA was preferably bound by AEX chromatography media, when similar amounts of HCP and DNA were spiked in the load material.

Characterization of the DNA size distribution in the load and the flowthrough fractions suggested preferential binding of larger (and therefore less safe) DNA fragments, further supporting the AEX chromatography capability to control DNA, independently of being a resin or membrane.

Overall, the AEX chromatography showed robust DNA clearance under a wide range process parameters and competing conditions for binding, thus expanding the platform knowledge for this media. From a control strategy standpoint, this study supports the high level of assurance for DNA clearance by the AEX chromatography media, typically used as a polishing step in downstream processing, after other chromatography steps (typically a capture and/or polishing) The use of AEX chromatography media in purification processes for biopharmaceuticals as a flowthrough provides step clearance redundancy and assurance that DNA can be cleared to the WHO acceptable safety level, while predominantly clearing larger, potentially oncogenic DNA fragments.

Acknowledgment

The authors thank Robert Barlow for providing analytical supports on DNA testing and preparation of DNA stock.

Literature Cited

1. Petricciani J, Loewer J. An overview of cell DNA issues. *Dev Biol*. 2001;106:275–282.
2. Sheng L, Cai F, Zhu Y, Pal A, Athanasiou M, Orrison B, Blair DG, Hughes SH, Coffin JM, Lewis AM, Peden K. Oncogenicity of DNA in vivo: tumor induction with expression plasmids for activated H-ras and c-myc. *Biologicals*. 2008;36:184–197.
3. Sheng-Fowler L, Lewis AM, Peden K. Issues associated with residual cell-substrate DNA in viral vaccines. *Biologicals*. 2009; 37:190–195.
4. *WHO Technical Report Series No. 941*. Geneva, Switzerland: WHO Expert Committee on Biological Standardization; 2007.
5. Fasullo FJ Jr, Fritsche HA, Liu FJ, Hamilton RG. IgG heavy chain subclass typing of myeloma paraprotein by isoelectric focusing (IEF) immunoblot analysis. *Clin Chem*. 1989;34:364–368.
6. Lehermayr C, Mahler HC, Mäder K, Fischer S. Assessment of net charge and protein-protein interactions of different monoclonal antibodies. *J Pharm Sci*. 2011;100:2551–2562.
7. Strauss DM, Cano T, Cai N, Delucchi H, Plancarte M, Coleman D, Blank GS, Chen Q, Yang B. Strategies for developing design spaces for viral clearance by anion exchange chromatography during monoclonal antibody production. *Biotechnol Prog*. 2010; 26:750–755.
8. Iskra T, Sacramo A, Gallo C, Godavarti R, Chen S, Lute S, Brorson K. Development of a modular virus clearance package for anion exchange chromatography operated in weak partitioning mode. *Biotechnol Prog*. 2015;31:750–757.
9. Phillips M, Cormier J, Ferrence J, Dowd C, Kiss R, Lutz H, Carter J. Performance of a membrane adsorber for trace impurity removal in biotechnology manufacturing. *J Chromatogr A*. 2005;1078:74–82.
10. Bhut BV, Weaver J, Carter AR, Wickramasinghe SR, Husson SM. The role of polymer nanolayer architecture on the separation performance of anion-exchange membrane adsorbers: part II. DNA and virus separations. *Biotechnol Bioeng*. 2011;108:2654–2660.
11. Woo M, Khan NZ, Royce J, Metha U, Gagnon B, Ramaswamy S, Soice N, Morelli M, Cheng KS. A novel primary amine-based anion exchange membrane adsorber. *J Chromatogr A*. 2011;1281:5386–5392.

12. Parra SC, Gebski C. Benefits of a revised approach to anion exchange flow-through polish chromatography. *Biopharm Int*. 2011;4:16–20.
13. Butler MD, Kluck B, Bentley T. DNA spike studies for demonstrating improved clearance on chromatographic media. *J Chromatogr A*. 2009;1216:6938–6945.
14. Iznaga AB, Frontera MC, Uramis JR, Gonzalez JBT, Parra YC, Gonzales YM. DNA removal from a purification process of recombinant hepatitis B surface antigen. *J Biotechnol*. 2007;10: 38–47.
15. vanReis R, Zydney A. Review bioprocess membrane technology. *J Membr Sci*. 2007;297:16–50.
16. Jin M, Szapiel N, Zhang J, Hickey J, Ghose S. Profiling of host cell proteins by two-dimensional difference gel electrophoresis (2D-DIGE): implications for downstream process development. *Biotechnol Bioeng*. 2010;105:306–316.

Manuscript received May 12, 2017, and revision received Aug. 29, 2017.