Clearance of Murine Leukemia Virus (MuLV) From a Chimeric Monoclonal Antibody Using Ion Exchange Chromatography

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Abstract

C225 (also known as Cetuximab) is a chimeric monoclonal antibody (MAb) with specificity for the human epidermal growth factor receptor (EGFr). C225, grown in serum-free production medium, is currently undergoing clinical trials. Maloney ectropic murine leukemia virus (Maloney-MuLV) was chosen as the model virus for the initial virus clearance validation of the C225 purification process. MuLV is an enveloped retrovirus, approximately 80 to 110 nm in size. The purification process, a five-step chromatographic procedure, utilizes Macro-Prep® High Q anion and Macro-Prep High S cation exchange supports. For the validation, conducted at an off-site location, load samples were spiked with known titers of MuLV. The spiked load samples were run on scaled-down versions of process chromatography columns. The spiked anion load had a titer of 1.9×10^6 plaque forming units (PFU)/ml and the cation load a titer of 5.75×10^5 PFU/ml. The anion exchange feed stream (10.6 ml, $10^{7.3}$ PFU total) was loaded onto the anion exchange support under pH and conductance conditions where C225 does not bind to the matrix and contaminants such as DNA and RNA bind strongly. The anion flow-through process intermediate was loaded onto the cation exchange support under conditions where C225 binds and is then eluted (10.6 ml, $10^{6.8}$ PFU total. To determine the amount of viral reduction, samples were taken of the load, flow-through (anion exchange step) and eluate (cation exchange step) for each run. The virus level in the samples was quantified by XC plaque-forming activity. The entire purification scheme netted a total of 15 $\log_{10}$ of MuLV clearance. The two ion exchange supports in combination resulted in a total of a nearly 8 $\log_{10}$ reduction of MuLV. The anion exchange step gave a 5.7 $\log_{10}$ reduction of the viral load and the cation exchange step a 2 $\log_{10}$ reduction.

Introduction

Virus validation with a model virus (or viruses) demonstrates to what extent a purification scheme is capable of reducing and/or removing viral particles that may be present in a cell line producing a biologic. Demonstrating potential virus inactivation and/or removal via the production process is recommended in the production of biologicals (US FDA 1993). The number and type of viruses used in a validation study depends upon many factors, including the type of cell line the product is from, and the intended use of the product (US FDA 1993). Virus validation is usually performed after a purification scheme has been developed and scaled up, so it is important to consider matrices that remove contaminating viruses (and nucleic acids in general) from the feed stream during the development of the purification. The ramifications of the chosen chromatographic steps should be evaluated for the positive implications on the virus validation study.

At an off-site location, a scaled-down version of the C225 production process was tested with spiked column loads. The initial virus clearance validation of the C225 purification scheme was challenged with the model virus, MuLV, in order to show the ability of the process to reduce the viral particle load from the feed stream. MuLV was chosen as the model virus because the antibody is of murine origin, and MuLV may potentially be present (US FDA 1993). A baseline level of starting viral load in the feed was determined prior to the virus study. In general, an overall target viral reduction due to purification should be greater than the determined prepurification viral load (US FDA 1993). As a general guideline, 3 to 4 $\log_{10}$ of reduction beyond the starting viral titer is a good safety margin.

Methods

All buffers used in this study were made prior to the validation study in an aseptic manner using USP-grade chemicals and water for injection. The solutions were 0.2 µm filtered and stored in sterile containers until the start of the virus validation study. The buffers were tested prior to the study for potential interference in the virus plaque assays. None of the buffers caused interference at the working assay concentrations.

All load samples, partially purified intermediates, for each the columns were generated before the validation study started and stored at appropriate conditions. The anion and cation loads were 12 ml each. Prior to the chromatographic run, 0.6 ml of the MuLV spike was added to the load sample.

The chromatography columns were packed with Macro-Prep High Q anion exchange support and Macro-Prep High S cation exchange support (Bio-Rad Laboratories, Hercules, CA).

The anion exchange column was preequilibrated and equilibrated at 750 cm/hr (588.7 ml/hr, 9.8 ml/min). Loading and running was done at 300 cm/hr (235 ml/hr, 3.9 ml/min). Two 1 ml samples of the spiked load were taken to determine the baseline or starting virus load, so the actual load volume was approximately 10.6 ml. After the column was equilibrated, the sample was loaded. Collection of the anion flow-through started at approximately 2% of full-scale absorbance. Upon completion of the load, the anion exchanger was washed with additional volumes of equilibration buffer until the OD 280 was back to baseline. When the OD 280 reading was approximately 5%, the sample flow was directed to waste.

The Macro-Prep High S cation exchange column was preequilibrated, equilibrated, and stripped at 750 cm/hr (588.7 ml/hr, 9.8 ml/min). Loading was done at 300 cm/hr (235 ml/hr, 3.9 ml/min). The product was eluted from the column at a flow rate of 50 cm/hr (39.3 ml/hr, 0.6 ml/min). Two 1 ml samples of the spiked load were taken to determine the baseline or starting virus load, so the actual load volume was approximately 10.6 ml. After the column was equilibrated the sample was loaded. The column was washed with load buffer for approximately 10 column volumes. After the wash step, the flow rate was decreased and the bound antibody was eluted and collected at ascending FS absorbance and 5% FS descending absorbance.

The presence of virus was assayed using the XC plaque-forming assay. SC-1 cells are used in the assay due to their sensitivity to MuLV. Several dilutions of samples of interest were made and introduced to a plate of SC-1 cells. The plaques that formed were counted and a determination of the PFUs was concluded.

Results and Discussion

The anion exchange load contained a total of 7.3 PFU $\log_{10}$ (Table 1). The anion step control sample had a total of 7.1 PFU $\log_{10}$ and the anion flow-through contained 1.6 PFU $\log_{10}$. In total, the anion exchange step cleared 5.7 PFU $\log_{10}$ of MuLV. Figure 1 shows the chromatography profile for the anion exchange step.

Table 1. MuLV Clearance Using Anion and Cation Exchange Chromatography

Sample	Volume (ml)	Titer (PFU/ml)	Total PFU ($\log_{10}$)
Anion load	10.6	1.9×10^6	**7.3**
Anion flow-through	21.5	<2.0	**1.6**
Anion control	10.6	1.2×10^6	7.1
Spiking virus	0.53	7.6×10^7	7.6
Anion Exchange Step Clearance			**5.7**
Cation load	10.6	5.8×10^5	**6.8**
Cation eluate	4.7	1.2×10^4	**4.8**
Cation control	10.6	7.2×10^5	6.9
Spiking virus	0.53	7.6×10^7	7.6
Cation Exchange Step Clearance			**2.0**

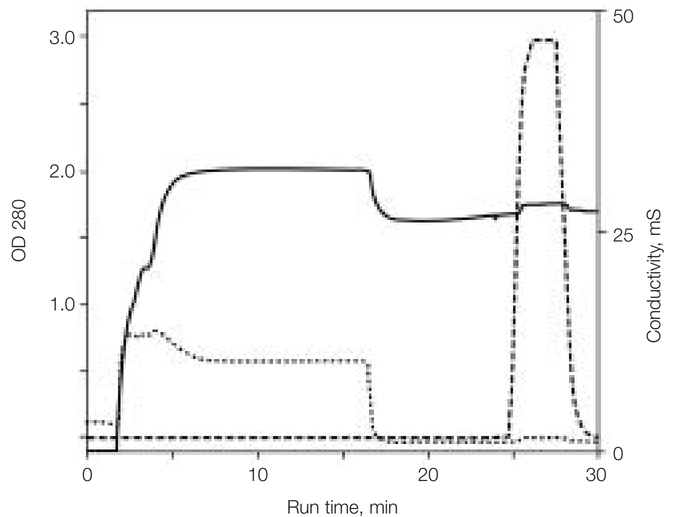

Fig. 1. The anion exchange step. The sample is passed over the column without being adsorbed, while the MuLV is adsorbed. In this step, 5.7 $\log_{10}$ of MuLV were removed.

The cation exchange load contained a total of 6.8 PFU $\log_{10}$ (Table 1). The cation step control sample had a total of 6.9 PFU $\log_{10}$. After the cation purification step, a total of 4.8 PFU $\log_{10}$ was present in the cation eluate (Table 1). The overall MuLV clearance at this step was 2.0 PFU $\log_{10}$. Figure 2 shows the chromatography profile of the cation exchange step.

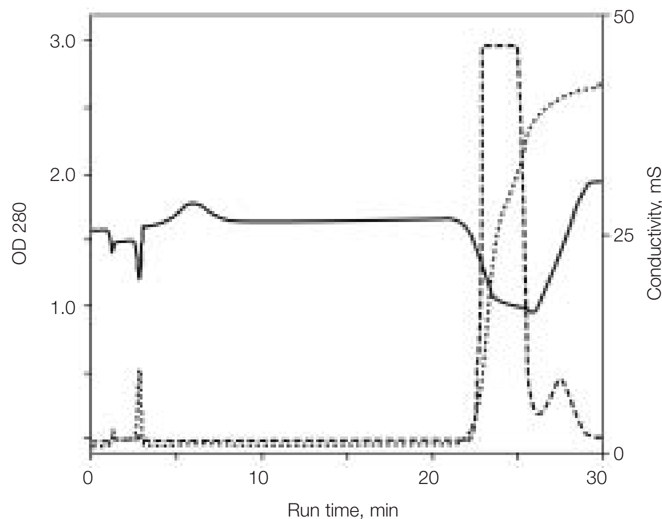

Fig. 2. The cation exchange step. The sample is adsorbed to the column while contaminants flow through. In this step, 2 $\log_{10}$ of MuLV were removed.

During the study, a second peak eluted off the cation exchange column after the main antibody peak. Upon additional analysis (gel electrophoresis), it was determined that 3% of the C225 antibody was present in the second peak. This relatively small second peak had not been part of the elution profile in previous purifications and was not anticipated. When the cation load without a virus spike from the same bulk load sample used in the viral clearance study was run on the cation exchanger, no second peak appeared. It is concluded that the second peak was generated by components in the virus spike interacting with the antibody.

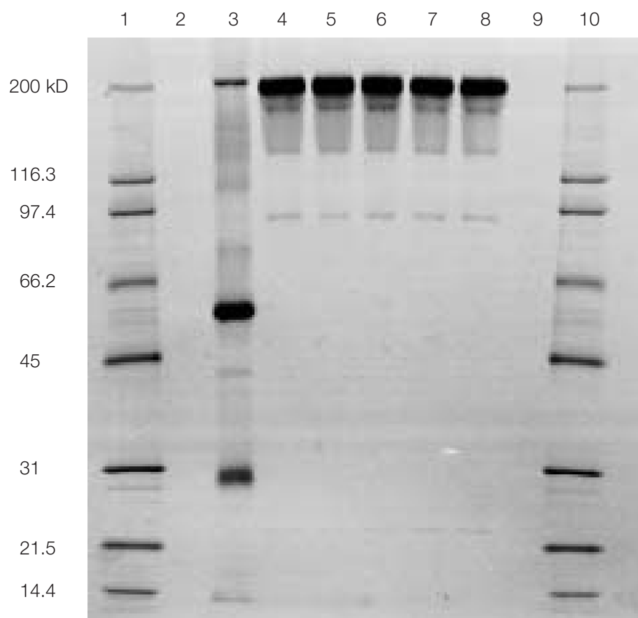

Fig. 3. Nonreduced SDS-PAGE. Silver-stained 4–20% gel. Lane 1, MW markers; 3, 10x concentrated sample; 6, anion pool; 7, cation pool; 10, MW marker. Total protein, 2 µg in each well.

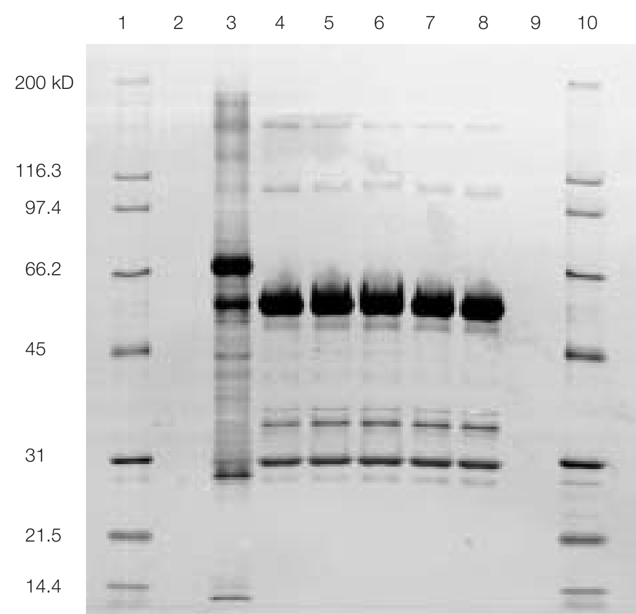

Fig. 4. Reduced SDS-PAGE. Silver-stained 4–20% gel. Lane 1, MW markers; 3, 10x concentrated sample; 6, anion pool; 7, cation pool; 10, MW marker. Total protein, 2 µg in each well.

Silver-stained SDS-PAGE analysis of the anion flow-through fraction and the cation exchange eluate showed about 95.55% and 96.4% monomer respectively (Figure 3).

The reduced gels for anion and cation were both about 95.6% heavy and light chain (Figure 4).

Summary

The combination of the Macro-Prep High Q strong anion exchange support and Macro-Prep High S strong cation exchange support resulted in a 7.7 $\log_{10}$ overall reduction of MuLV. The two columns together in the C225 purification scheme significantly contributed to the inactivation/removal capacity of the purification process.

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Reference

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