Simplifying Progress

Efficient Removal of Activated Coagulation Factor XI (FXIa) from Cohn Fraction II Paste Using Mixed Mode Ligand

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1. Introduction

Factor XI (FXI) is the zymogen of a serine protease, Factor XIa (FXIa). It is a 160 kDa protein, with an average plasma concentration of 3 to 7 µg/ml. Its presence in IgG preparations is associated with the risk of thromboembolic events in patients. FXI poses challenges due to its (auto) activation during the fractionation process under certain conditions. Intravenous administration of the IgG-containing FXIa has been shown to induce activation of the intrinsic coagulation pathway and cause blood clot formation, posing a life-threatening risk to patients.

During the cold ethanol plasma fractionation process, some of the coagulation factors, for example, factors II, IX, and X are removed from IgG, but FXI remains a challenge because of its co-precipitations with IgG. The aim of this study was to evaluate the removal of FXIa from Fraction II paste (containing mainly IgG) using a chromatographic monolithic column, modified with a novel multimodal ligand, as an alternative to affinity-based resin chromatography. The multimodal chromatographic purification approach was proven to selectively bind FXIa. In addition, the process parameters, such as temperature and flow rates, were optimized for FXIa removal to define the process parameters for scaling up.

2. Experimental Approach

Fraction II paste obtained via the modified Cohn method, provided by National Bioproducts Institute NPC (Pinetown, South Africa), was resuspended in purified water at a ratio of 7 L water/kg paste, clarified, adjusted, filtered and used for the experiments 20 mg/ml IgG concentration. The suspension contained approx. 1000 mIU/mL FXIa concentration (sample). Chromatography runs in negative mode (IgG was in flow-through fractions) were performed on CIMmultus multimodal-based monolith columns, ranging from bed volumes of between 1 and 400 mL and 0.2 mL monolith unit (2 µm channels) (Sartorius BIA Separations, Slovenia). The small non-GMP 0.2 mL monolith unit (Specimen) was extracted from the same batch of bulk monolith as its parental column (Figure 1). 1 mL CIMmultus columns as the smallest preparative scale were used for method and process optimization, before transferring the process on preparative CIMmultus format.

Experiments were performed at room temperature (RT) or at a controlled temperature of 6 °C. After column equilibration, from 60 column volume (CV) up to 122 CV, the sample was loaded, followed by washing, elution and cleaning in place with a solution of 0.5 M NaOH and 2 M NaCl for 20 minutes. IgG concentration and recovery in preparative chromatography fractions were determined using the Atellica NEPH 630 nephelometer (Siemens Healthineers, Germany). FXIa in the samples was quantified using a chromogenic kit Rox Factor XIa (Rossix, Sweden) with a limit of detection of about 0.03 mIU/mL FXIa.

The buffers compositions were systematically optimized by evaluating the effects of various parameters on FXIa removal performance. The factors investigated included buffer composition, pH, conductivity, and the presence of different additives. The specific mobile phase compositions utilized in this study are confidential and therefore not disclosed.

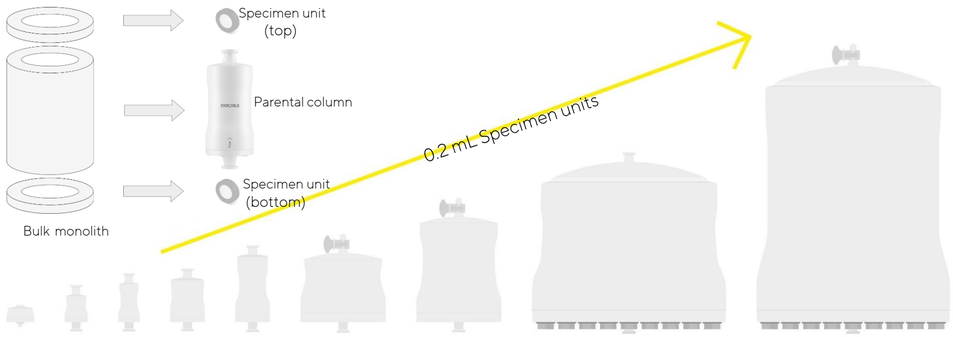


Figure 1: Schematic presentation of the parental columns and their extracted 0.2 mL Specimen units. After monolith production, excess material is cut from the parental monolith, and the parental monolith is packed as a CIMmultus column. Specimen units from positions above (top) and below (bottom) of the parental monolith are packed as 0.2 mL Specimen monolith units. Therefore, the monolith material composition of Specimen and parental monolithic columns are identical.

3. Results

Determination of FXIa binding capacity

After optimizing the buffers, a total of 122 CV of the sample were loaded onto the 1 mL CIMmultus column. Flow-through was collected in 5 mL fractions, and the FXIa activity concentrations were determined in each fraction. Cumulative concentrations for FXIa were calculated for each volume. Additionally, pooled flow-through of 40 CV, 75 CV, and 122 CV of the initial sample were analyzed for FXIa concentration and IgG recovery for the entire volume of the 122 CV. The concentration results of FXIa from the measured fractions and their cumulative values are presented in Figure 2, while the results from the pooled 40 CV, 75 CV and 122 CV fractions are summarised in Table 1.

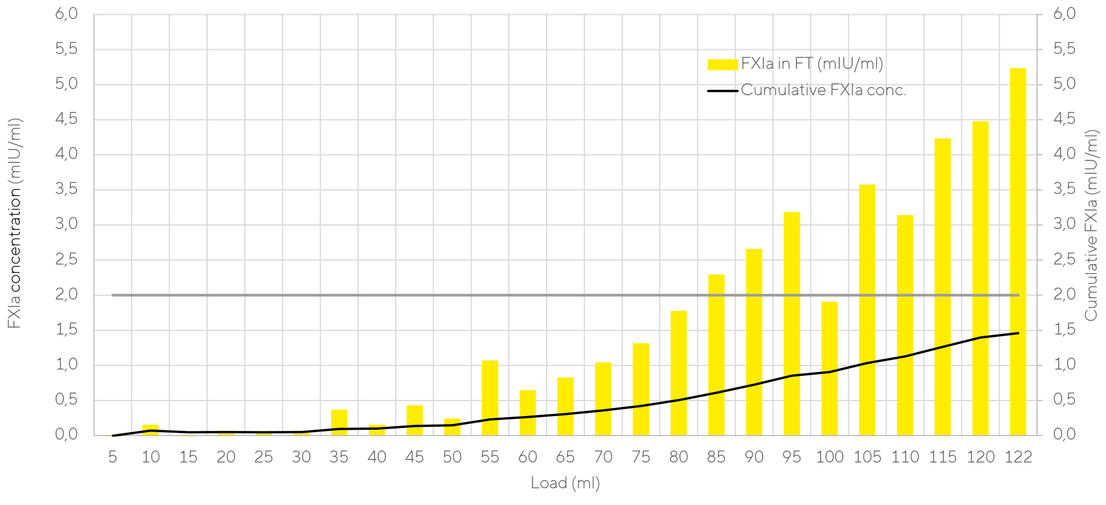


Figure 2: FXIa activity concentration in the flow-through IgG fractions and cumulative FXIa activity concentration in the flow-through pool using 1 mL CIMmultus multimodal column.

The cumulative FXIa results shown in Figure 2 and the pooled flow-through of 40 CV, 75 CV, and 122 CV sample loads presented in Table 1 are below the specification limit < 2 mIU/mL.

Table 1: FXIa concentration in pooled flow-through IgG fractions (40 CV, 75 CV and 122 CV) performed on 1 mL monolithic column.

FXIa in pooled 40 CV sample (mIU/ml)	FXIa in pooled 75 CV sample (mIU/ml)	FXIa in in pooled 122 CV sample (mIU/ml)	IgG Recovery in pooled 122 CV sample (%)
0.1	0.4	1.6	100.0

Influence of temperature and flow rate on FXIa removal

The influence of flow rate on FXIa removal was tested using 1 mL CIMmultus column with optimized buffers at 0.25 CV/min, 0.5 CV/min, 1 CV/min, 2 CV/min and 5 CV/min. Although higher flow rates result in a slight decrease in the binding capacity of FXIa, the outcomes are still reliable, and the results indicate efficient mass transfer between the mobile and stationary phase using CIM monolithic column (Figure 3A). Experiments were also conducted at two temperatures, RT and at 6 °C, showing better results at RT compared to 6 °C (Figure 3B).

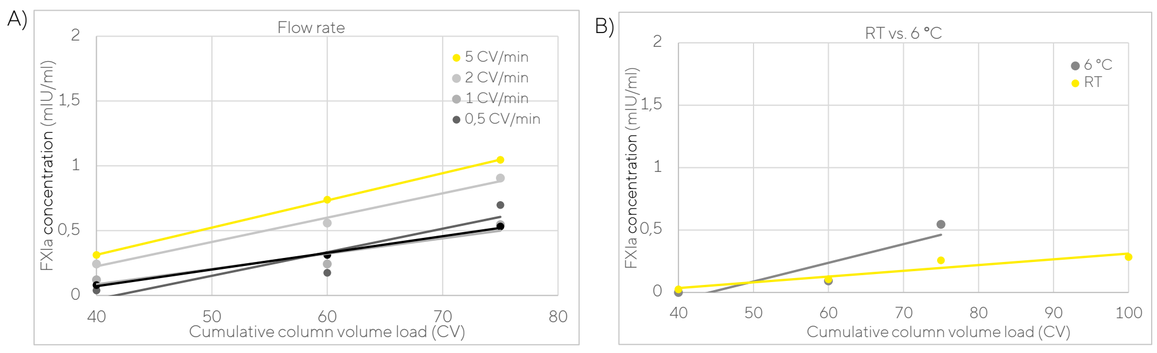


Figure 3: Influence of the flow rate (A) and temperature (B) on FXIa concentration in flow-through IgG fraction. Experiments were performed on 1 mL CIMmultus columns.

Intra-column Homogeneity and Batch-to-batch Reproducibility

The removal efficiency of FXIa was evaluated using both top and bottom Specimens (Figure 1) from a parental 80 mL CIMmultus column to demonstrate the homogeneity of larger-scale monoliths. The results were consistent, indicating the homogeneity of the monolith (Figure 4A). Furthermore, three different batches of 1 mL CIMmultus columns resulted in comparable FXIa removal efficiency (Figure 4B), confirming column batch-to-batch reproducibility.

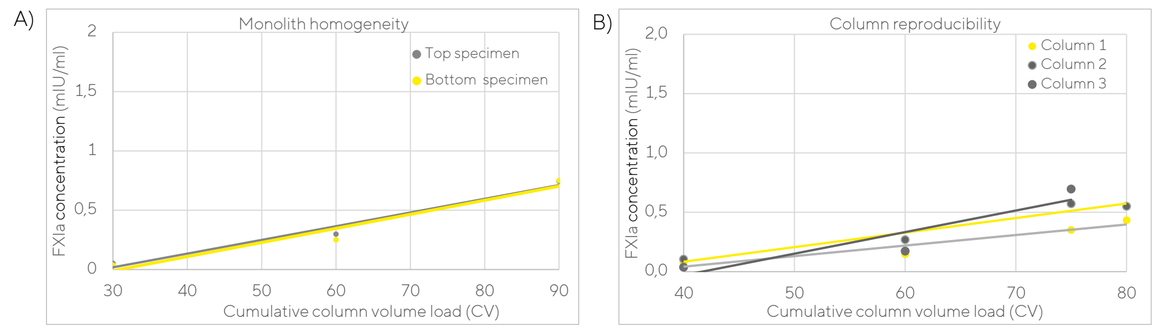


Figure 4: Remaining FXIa concentration in the flow-through IgG fraction determined on the top and bottom Specimen from parental 80 mL column (A) and on 1 mL monolithic columns from three different batches at 6 °C (B).

Impact of NaOH cleaning on column lifespan

Lifetime of 1 mL CIMmultus multimodal columns using 0.5 M NaOH and 2 M NaCl as cleaning agent was tested after 10 and 20 cycles at 6 °C (Table 2). No change in FXIa binding capacity after 20 cycles has been noticed.

Table 2: FXIa concentration in the flow-through IgG fraction determined on three different batches 1 mL monolithic column after 0, 10 and 20 cycle.

Monolith column	No. of cycles	FXIa in pooled 60 CV sample (mIU/ml)	FXIa in pooled 75 CV sample (mIU/ml)
Column 1	0	0.2	0.2
	10	0.3	0.4
	20	0.1	0.3
Column 2	0	0.1	0.4
	10	0.2	0.5
	20	0.2	0.4
Column 3	0	0.0	0.2
	10	0.2	0.3
	20	0.2	0.3

Scaling-up the purification process

To demonstrate the scalability of the process, a scale-up study was conducted using a 400 mL column, with 75 CV of the sample loaded at RT. Results from 1 mL and 400 mL columns confirmed the scalability of both the purification process and the columns (Table 3) .

Table 3: Remaining FXIa concentration and IgG recovery in the flow-through IgG fraction performed on the 1 mL and 400 mL monolithic column at RT.

Monolith	FXIa in pooled 75 CV sample (mIU/ml)	IgG recovery (%)
1 mL column	0.1	98.3 %
400 mL column	0.1	96.6 %

4. Conclusions

The study revealed that CIMmultus multimodal monolithic columns (2 µm channels) selectively bind and effectively remove FXIa from IgG fractions below specification limits. The data presented demonstrate that the use of the CIMmultus multimodal monolithic columns allows:

- High binding capacity of FXIa.
- Highly reproducible results.
- High IgG recovery.
- Efficient scale-up.
- Consistent results across all 20 CIP cycles in 0.5 NaOH and 2 M NaCl.

These results highlight the reliability and reproducibility of the process using CIMmultus multimodal column, ensuring consistent IgG production quality.

5. References

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