Capture of GFP on the new anion exchanger Capto Q

Intellectual Property Notice: The Biopharma business of GE Healthcare was acquired by Danaher on 31 March 2020 and now operates under the Cytiva™ brand. Certain collateral materials (such as application notes, scientific posters, and white papers) were created prior to the Danaher acquisition and contain various GE owned trademarks and font designs. In order to maintain the familiarity of those materials for long-serving customers and to preserve the integrity of those scientific documents, those GE owned trademarks and font designs remain in place, it being specifically acknowledged by Danaher and the Cytiva business that GE owns such GE trademarks and font designs.

cytiva.com

CY15119-11Jul20-PT

Capture of GFP on the new anion exchanger Capto™ Q

Anna Åkerblom, Kjell Eriksson, Mattias Bryntesson and Fariba Sabounchi-Schütt

GE Healthcare, Amersham Biosciences AB, Björkgatan 30, SE-751 84 Uppsala, Sweden

Summary

Capture of Green Fluorescent Protein (GFP), from clarified *E. coli* homogenate, using packed bed chromatography on the newly developed strong anion exchanger Capto™ Q is presented. Method screening and optimization were done on laboratory scale, after which the optimized method was scaled to pilot scale. Based on the results obtained on laboratory and pilot scales, scale-up modelling and productivity calculations were done. The results show that it is possible to design a capture step where 100 kg or more of a protein, such as GFP, can be processed in less than 24 hours.

Background

Recent developments in upstream processing have resulted in increased protein expression levels and larger feed volumes. Capto Q is designed to meet this challenge by allowing increased speed and throughput in order to increase productivity and reduce process cycle times, particularly important in a capture step. The versatility of the medium stems primarily from its high rigidity and dynamic binding capacity. The high rigidity of the medium translates into the possibility to use high flow velocities and/or high bed heights while the high capacity gives the possibility to use columns with smaller volumes.

The goal of the study was to show that it is possible to capture 100 kg of product per 24 hours using Capto Q.

As a model substance recombinant Green Fluorescent Protein (GFP) was used. That is a protein with a M_W of 28 kDa with a pI of 6.2. One advantage using GFP as a model protein is its absorbance at 490 nm, which can be used as a simple assay. The protein was expressed in *E. coli*. In nature GFP can be found in the Pacific jellyfish *Aequorea victoria,* see Fig. 1. GFP has found a widespread use, including cell based assays.

Figure 1. The Pacific jellyfish, *Aequorea victoria.*

Experimental and Results

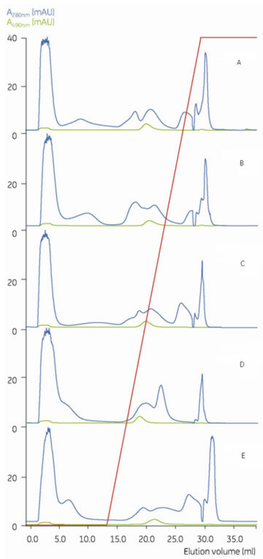


Figure 2. Screening of media selectivity. A is Capto Q, B is Q Sepharose XL, C is Q Sepharose Fast Flow, D is DEAE Sepharose Fast Flow and E is ANX Sepharose Fast Flow (hs). Blue curve: A280, Green curve: A490 (GFP).

Selectivity of different anion exchangers was screened using pre-packed 1 ml HiTrap™ columns. Media tested were Capto Q, Q Sepharose™ XL, Q Sepharose Fast Flow, DEAE Sepharose Fast Flow and ANX Sepharose 4 Fast Flow (hs). Screening was done in a 50 mM Tris/HCl buffer, pH 8.2. For elution; 15 CV gradient from 0 to 1 M NaCl, with a flow of 1 ml/min. Figure 2 shows the results from this screening. Capto Q was the medium that gave the highest resolution of GFP from the contaminants and was thus chosen for the additional screening and optimization.

Binding pH and elution conditions were optimized. This screening and optimization were done using Tricorn™ 5/100 GL columns (2ml CV); the optimal pH was found to be 8.2 using a 50 mM Tris/HCl buffer. Elution was performed with a stepwise elution with NaCl.

The optimized conditions were used when the method was scaled up to a FineLINE™ 70 column (808 ml CV). Figure 3 shows a comparison of the results obtained on the two scales. Only Capto Q was scaled-up. The results obtained on the two scales were the same; the purification factor and yield kept constant. Yield were on both scales, and in all experiments run, above 90%.

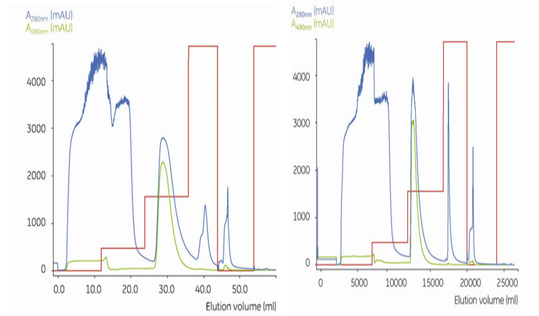


Figure 3. Capture of GFP on small scale (2ml) and pilot scale (808 ml). On small scale 30 mg and on pilot scale 12 gram of GFP was loaded per run.

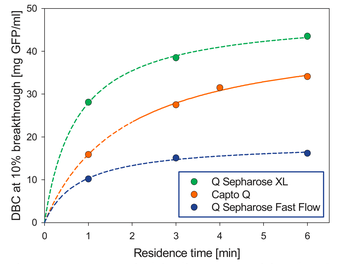


Figure 4. Dynamic binding capacities for GFP on Capto Q, Q Sepharose XL and Q Sepharose Fast Flow.

Dynamic binding capacities for GFP were determined at different residence times on Capto Q, Q Sepharose XL and Q Sepharose Fast Flow. Results shown in Fig. 4.

Scale-up calculations

Based on the results in Fig. 3 and the chromatography cycle data of the three media, productivity calculations for the three media were done.

Small scale experiments, using Capto Q, were run at 300 cm/h in a column with 10 cm bed height. A theoretical (for Capto Q also practical pilot scale) scale-up was done to 20 cm bed height, thus 600 cm/h (200 cm/h for Q Sepharose FF and XL) is used to keep residence time constant. Exceptions are the loading step, run at different flow velocities, and the CIP which is kept constant at 30 minutes. The cycle time when using Capto Q is thus approx. 1 ½ hours and for Q Sepharose XL and Q Sepharose Fast Flow the cycle times are approx. 3 ½ hours. The loading volume was assumed to be 70% of DBC (QB10%) and yield measured to be 93.5%. Varying the loading velocity gives the result shown in Figure 5. The high productivity achieved on Capto Q comes from both a high dynamic binding capacity and the possibility to use high flow velocities, even at large scale. From the figure it is seen that the goal set up, capture 100 kg of product per 24 hours using Capto Q, can be achieved. The column dimension needed to reach this productivity is 0.2 x 1.6 (i.d.) metre.

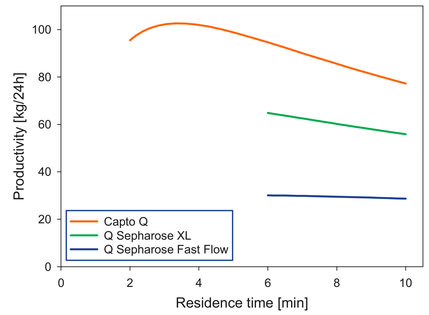
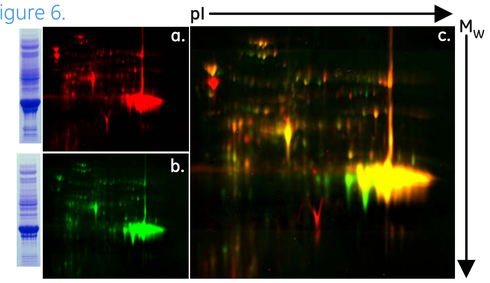


Figure 5. Comparison of calculated productivity on Capto Q compared to Q Sepharose XL and Q Sepharose Fast Flow.

Verification of scale-up

SDS-PAGE and 2D-electrophoresis was run on the eluates obtained from the two different scales. Results are shown in Figure 6.



Figures 6a. and 6b. show the results from 1D- and 2D-electrophoresis run on pilot scale and small scale, respectively. 6c. shows an overlay of the two 2D-electrophoresis gels. An almost exact match was obtained.

Conclusions

The results presented herein show that Capto Q gives a very high productivity when used for capture of a recombinant protein, in this example GFP expressed in *E. coli*. The combination of high volume throughput and high dynamic binding capacity makes Capto Q an attractive choice for processing large volumes and large amount of protein.

imagination at work