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Single Step Clarification & Capture of a Recombinant Protein from *E. coli* Osmotic Shock by Crossflow Chromatography

AN1000

Summary

- Recombinant Shiga toxin beta subunit was released from *Escherichia coli* following osmotic shock creating an osmotic shock fluid (lysate) and captured using a Natrix Cross Flow Chromatography step
- The number of steps in a conventional downstream capture purification process were reduced from three to one.
- Process yields were effectively raised from 80% to 95%, and at the same time process time was reduced from 12 hours to 1.5 hours with a reduction in elution volume by 67%.
- Overall production costs were significantly reduced.

Introduction

Purification of a recombinant protein is by definition a multi-step process.[1]

A typical downstream processing train for an intracellular product would normally include:

- an initial clarification step to separate the cells from the fermentation or cell culture broth,
- re-suspension and lysis of the cells **by osmotic shock,**
- a second clarification step to separate solubilised target protein from cell debris,
- normal flow filtration to clarify the feed stream in preparation for Primary Purification,[2]
- several resin chromatographic steps.

Most purification processes include a minimum of three resin chromatographic steps, one of which is usually the rate limiting step in a downstream process. Combining traditionally separate processing steps result in higher yields, reduced processing times, reduced labour and operating costs and reduced capital expenditure.[3]

Natrix chromatography membranes combine all the advantages of chromatographic resins (specificity, selectivity, high binding capacities) and conventional membranes (high throughput, ease of operation, scalability) in a single use format. Since Natrix membranes are inherently hydrophilic they are naturally resistant to fouling, and the Crossflow device formats permit simultaneous Clarification and Capture of a target molecule from an unclarified feed stream (fermentation or cell culture broth, cell lysate, allantoic fluid).

This application note describes the successful reduction of the total number of processing steps required to purify a recombinant protein produced intracellularly in Escherichia coli, through the introduction of a Natrix Crossflow Chromatography unit operation in place of the conventional multi-step Clarification & Primary Purification process.Chromatography unit operation in place of the conventional multi-step Clarification & Primary Purification process.

Materials and Methods

Figure 1: Shiga Toxin Beta Subunit

The Institut Pasteur in Paris, France has developed a novel vaccine delivery vehicle consisting of the beta subunit of the Shiga toxin molecule. The beta subunit is a 7.7 kDa pentameric protein (see Figure 1) that binds to specific glycolipids on host cells to provide targeted delivery of vaccine antigens.[4] In this work, Professor Carl Lawton, Director of the Massachusetts Biomanufacturing Center, in collaboration with the Institut Pasteur developed an optimized downstream process for purification of the recombinant subunit molecule produced by a proprietary strain of *E. coli*. In the conventional process, after fermentation the cells are harvested via centrifugation then re-suspended in lysis buffer. The cell lysate contains lipids, carbohydrates, nucleic acids, cells and cellular debris with a particle size of 0.5 – 1.5 microns. Total protein concentration is 2.2 mg/ml, with the Shiga toxin molecule constituting only 10% of total protein content. This complex mixture is typically centrifuged, then filtered through 0.8, 0.4 and 0.2 micron filters in series to remove any residual cell debris. The clarified stream is then applied to an Anion Exchange (Q) resin for primary purification, following which the purified eluate undergoes intermediate purification and final polishing to achieve the target purity. In this process the post-lysis clarification and capture steps each have yields of ~90-98% for a net yield of 80%, and the purity of the product after primary capture via Anion Exchange is 18%.

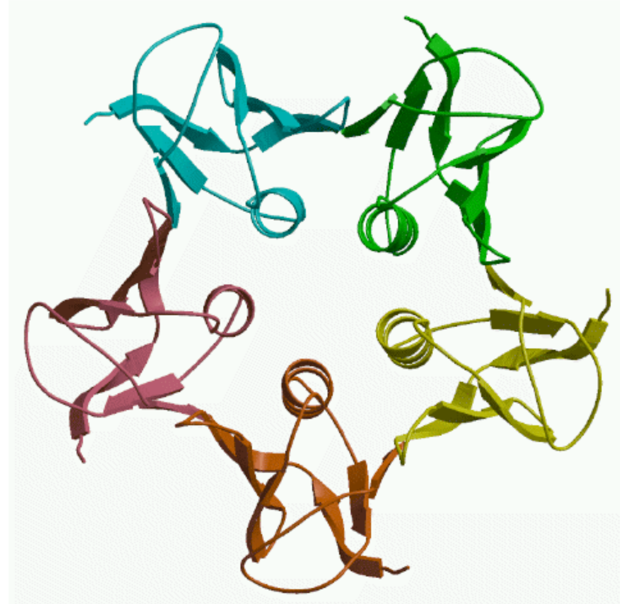

Figure 1 : Shiga Toxin
Randy J Read
University of Cambridge 2002
Image used by permission

In this work, as shown in Figure 2 below, the post-lysis centrifugation, filtration and Anion Exchange resin chromatography steps were replaced by a single Natrix Crossflow Chromatography step. The Natrix Crossflow Chromatography unit operation consisted of a spiral wound element containing Natrix *Adsept*™ Q, Anion Exchange membrane. Lysate was loaded onto the Natrix *Adsept*™ Q spiral in Tangential Flow (TFF) mode. The lysate was recirculated until the membrane was fully saturated. Conventional column chromatography does not allow recirculation of sample and capacities are typically well below saturation levels. The spiral was then washed and the target molecule eluted using the standard wash and elution buffers as in the conventional process. The concentration and purity of the eluate was determined using SDS-PAGE and HPLC analysis.

Figure 2: Conventional Downstream Process versus Optimized Process Utilizing Natrix Product.

Conventional Process

Fermentation → Centrifugation → Cell Lysis → Centrifugation → Filtration → Anion Exchange → Intermediate Purification

12h duration
80% yield
18% purity
3L elution vol.

Natrix Streamlined Process

Fermentation → Centrifugation → Cell Lysis → Natrix Q Spiral → Intermediate Purification

1.5h duration
95% yield
18% purity
1L elution vol.

Results and Discussion

In the conventional process, the net yield over the Clarification and Primary Capture steps post-lysis (i.e. centrifugation, serial filtration and resin bead Anion Exchange chromatography) was 80%. The purity of the target molecule after these processing steps was 18% and the actual processing time was 12 hours. The total time required to complete the Clarification and Capture steps in the conventional process including hold times was three days. This resulted in loss of biological activity due to proteases present in the sample. In the Natrix process, a single unit operation employing a Natrix *Adsept*™ Q spiral replaced the centrifugation, serial filtration and resin bead chromatography steps. This streamlined process was able to achieve the same level of clarification and purity (18%) as the conventional process, with a significantly higher yield (see Table 1). In addition, the processing time required to achieve Clarification and Primary Capture of the target molecule was reduced from 12 hours to 1.5 hours, eliminating the need for any overnight holds and reduced the loss of biological activity due to proteases. Further, in the conventional process the elution volume after the anion exchange chromatography step was 3L, while in the Natrix streamlined process the same quantity and purity of the target molecule was eluted in only 1L. The decrease in eluate volume improved the efficiency of the intermediate purification process as the sample volume was now reduced by 67%.

Table 1: Comparison of Product Yield, Purity and Total Processing Times for the Conventional vs Optimized Downstream Processes.tography

	Conventional Process	Natrix Optimized Process
Yield	80%	90%
Purity	18%	18%
Processing Time	12 Hours	1.5 Hours
Elution Volume	3 Litres	1 Litre

Conclusion

As demonstrated in the application described above, there are significant performance and economic advantages associated with the implementation of a simultaneous Clarification and Capture unit operation using Natrix Crossflow technology. These include reduced processing times, improved yields and reduced elution volumes, all of which have a significant positive impact on the cost of goods to produce a given target molecule.

For more information on *Adsept*™ membrane chromatography products, please contact Natrix Separations Inc., or visit our website: www.natrixseparations.com

Ordering Information

Device Format	Product Description	Chemistry	#Catalog
Casette	0.01 SQM Mini Cassette	Q	TCQAU1A2S
Casette	0.02 SQM Mini Cassette	Q	TCQAU2A2S
Casette	0.05 SQM Mini Cassette	Q	TCQAU5A2S
Casette	0.10 SQM Mini Cassette	Q	TCQAUAA2S
FP Spiral	2” x 6” FP Spiral	Q	FCQA2006A4
FP Spiral	2” x 6” FP Spiral	Q	FCQA3810A4

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