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Refolding buffer clarification

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ABSTRACT

Protein refolding is the phenomena by which the inactive protein is translated into its native form generally by folding in dilution buffer. The dilution buffer often needs to be clarified before subjecting to further purification processes. The key challenge in the pre-filtration step is to minimize the product loss, obtain desirable filtrate quality to enhance the efficiency of subsequent purification processes. Cellulose based depth filters are widely used in biopharmaceutical industry for this application. Zeta plus™ EXT SP series - dual zone depth filter grades were screened to ascertain the clarification performance. The results showed that Zeta plus EXT 60SP02A could deliver optimum throughput and flux, and desirable filtrate quality required to protect the downstream equipments such as chromatographic columns and thereby bringing down the overall filtration costs and downtime in the scale-up.

KEYWORDS

Protein refolding;
Depth filters;
Zeta plus;
Throughput;
Flux;
Scale-up.

INTRODUCTION

Protein refolding

Recombinant DNA technology paved path for the introduction of a wide range of biopharmaceuticals in the market. The rDNA technology is markedly prevalent for protein expression in *E.Coli* and yeast. The expression of recombinant proteins in *E.Coli* often results in the formation of insoluble and inactive protein aggregates commonly known as inclusion bodies. Generally, four steps are followed to get the active form of protein from these protein aggregates. In the first step, the bacterial cells are lysed and the protein aggregates are collected by centrifugation. In the second step, the cell wall and outer membrane components are removed from the protein aggregates. In the third step, the protein aggregates are solubilized with strong protein denaturants. In the final step, the solubilized denatured proteins are folded with suitable refolding buffer to obtain the structure of native protein (1).

Clarification by depth filtration

Depth filters are generally used as pre-filters in the processing of biopharmaceuticals post harvest of the fermentation broth. Pre-filtration can be described as any filtration step incorporated into a manufacturing process prior to the final filtration. The usual purpose of pre-filtration is to remove contaminants from the process stream and, it is very important to incorporate one or more stages of pre-filtration to remove the particulate matter which will minimize loading on the final filter. Depth filtration is the preferred pre-filtration method employed where the contaminant loads are compara-

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tively higher. Cellulose based depth filter is one of the most preferred pre-filters in the biopharmaceutical processes. The basic raw materials used in these types of depth filters are cellulose, inorganic filter aids and anionic resins. Cellulose based filters can provide effective particle retention rating to a minimum of 0.1 μm. The retention rating of depth filters are referred as a nominal value which means that it will remove at least one particle of the stated size (2).

Zeta plus™ is a family of advanced cellulose-based depth filtration media designed to retain contaminants by both mechanical straining and electro-kinetic adsorption. Zeta plus multi cell cartridge filtration system combines high efficiency separations with extended filter life in a totally enclosed environment. The Zeta plus EXT series filter consists of two distinct layers of filter media with upstream zone coarser than the downstream zone. This structure enhances the contaminant loading capacity of the filter media since larger particles are trapped in the upper zone of the filter media and finer ones in the lower zone, reducing premature plugging and thus extending service life.

Depth filter positioning

The positioning of the unit operation in a bioprocess needs optimization with the process fluid in the bench scale and pilot scale before the production scale. A series of one or two small-scale filters (bench-scale and/or pilot-scale) is often studied when attempting to define production-scale filtration requirements. Capsule data should first be generated using the Zeta plus Biocap System, Biocap 25 (25 cm^2), and/or Biocap E03 (1020 cm^2). In critical applications, both Biocap 25 and Biocap E03 data should be generated. After collection of the data, production scale filtration area requirements can then be safely estimated considering engineering safety margins.

The general clarification method adopted for refolding buffer processing step includes combination of pre-filters viz. 5μm, 1.2μm, 0.45μm to attain the desired filtrate quality, further subjected to chromatographic steps including anionic exchange, hydrophobic interaction, size exclusion, and so on. Alternatively, cellulosic depth filters can be employed to combine multiple prefiltration steps into a single processing step thereby reducing the downtime, and cost of filtration, efficient protein recovery with minimized product loss and ease of operation.

Thus the cellulosic depth filtration method is considered to be the efficient method for clarification of refolding buffer in the industry.

Test procedure

The standard operating procedure for Biocap-25 was followed for the trial run at lab scale. The constant flow test method was adopted for the trial with a constant flux depending on the retention rating of the filter.

The differential pressure was monitored constantly to check for the pressure breakthrough. Optical density (OD) of the harvest and pooled OD of the filtrate was recorded at 600 nm to correlate the filter performance.

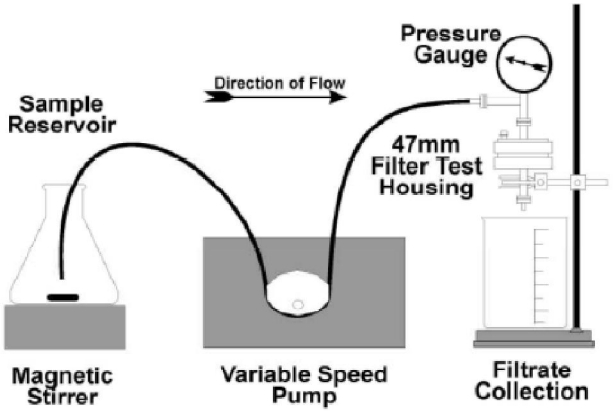

Figure 1 : A typical representation of constant flow apparatus

The Zeta plus grades in Biocap-25 configuration viz. 10SP02A, 30SP02A, and 60SP02A were screened to record the performance. The grade 60SP02A with a retention rating range of 0.7 μm to 0.2 μm gave an optimum throughput and filtrate quality to improve the efficiency of subsequent downstream processing steps.

RESULTS AND DISCUSSION

The trial results showed that the average throughput obtained from 60SP02A was approximately 200 Litre per square metre with a flux rate of 170 LMH. The resultant filtrate was subjected directly to HPLC to determine the protein recovery. The results showed a 95% product recovery with considerable reduction in the contaminant profile.

The chart (figure 2) below shows that there is per-

ceptible reduction on the particle load and excellent filtrate quality. The trend also shows that the there was

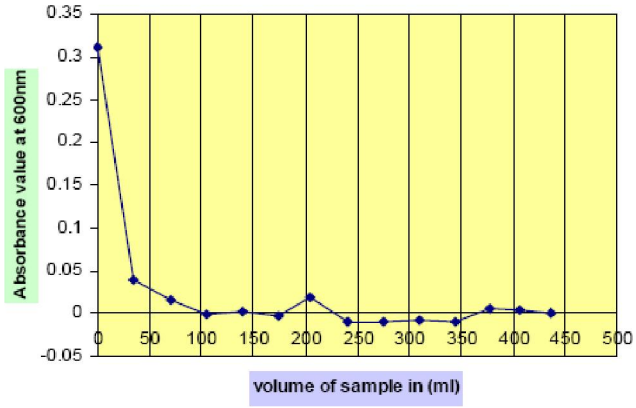

Figure 2 : Filtration chart for Zetaplus Biocap 25 EXT series with 60SP02A

no break of OD till the last sample collected.

The filter sizing for the production scale was arrived from the lab and pilot scale trial data.

SUMMARY

The Zeta plus EXT 60SP02A grade proved to be efficient for the refolding buffer clarification in offering protection to the purification column. The lab scale results were successfully scaled up and could demonstrate the consistency of the filtration performance.

REFERENCE

[1] Paul T.Wingfield, Ira Palmer, Shu-Mei Liang; Purification of recombinant proteins. Current Protocols in Protein Science. Second. New Jersey : John Wiley & Sons, Inc., **(1995)**.

[2] Maik W.Jornitz, Theodore H.Meltzer; Filtration and Purification in the Biopharmaceutical Industry. Second. New York : Informa Healthcare, **174**, 978-0-8493-7953-6 **(1998)**.

[3] J.Pasternak, Jack; Recombinant DNATechnology. An Introduction to Human Molecular Genetics: Mechanisms of Inherited Diseases. Second. New Jersey : John Wiley & Sons,Inc., 107-152 **(2005)**.

[4] R.K.Scopes; Overview of Protein Purification and Characterization. Current Protocols in Protein Science. New Jersey : John Wiley & Sons, Inc., **(1995)**.

[5] Exploitation of the Adsorptive Properties of Depth Filters for Host Cell Protein Removal during Monoclonal Antibody Purification. Yinges Yigzaw, Robert Piper, Minh Tran, Abhinav A.Shukla. 1, Washington : American Chemical Society, 2006, Biotechnology Progress, **22**, 288-296, 1520-6033.

[6] Purification of overproduced Escherichia coli RNA polymerase σ factors by solubilizing inclusion bodies and refolding from Sarkosyl. Burgess, Richard R.Madison : Elsevier Inc., Methods in Enzymology, **273**, 145–149 **(1996)**.