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Journal of Chromatography B, 784 (2003) 183–187
JOURNAL OF CHROMATOGRAPHY B
www.elsevier.com/locate/chromb

Short communication

Expanded bed adsorption processing of mammalian cell culture fluid: comparison with packed bed affinity chromatography

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Received 2 May 2001; received in revised form 8 July 2002; accepted 16 September 2002

Abstract

A comparison between expanded bed adsorption and conventional packed bed Protein A Fast Flow to purify the anti-rHBsAg mAbs from feedstock is presented in this work. Direct capture by STREAMLINE expanded bed adsorption chromatography resulted in 92% product recovery and sevenfold more concentrated product with similar purity levels compared to that obtained by the standard packed method. The process time and buffer consumption were reduced in the expanded bed adsorption method not only with the binding-elution conditions but also with the use of NaOH during the cleaning-in-place step. The latter is the most widely accepted agent in downstream processing, being a cost effective technique that provides not only efficient cleaning but also sanitizes complete column systems and destroys pirogens.

Keywords: Expanded bed adsorption; Monoclonal antibodies

1. Introduction

The use of affinity ligands in expanded bed adsorption (EBA) may improve its use in direct recovery operations, as the enhanced selectivity of the adsorbent permits selective capture of the target produced from complex feedstocks as well as high degrees of purification.

Around 10 years ago Draeger and Chase [1] reported the first stable fluidized (expanded) bed with chromatographic characteristics similar to a packed bed. The application of mixtures of proteins and cells onto these expanded beds showed the potential of this technique for the recovery of proteins from particle-containing feedstocks [2,3]. Draeger and Chase subsequently optimized these first results up the obtention of particles with higher sedimentation velocity to fully exploit the features of the expanded bed technology.

In 1993 Amersham Pharmacia Biotech introduced new types of chromatographic adsorbents and columns called STREAMLINE [4]. These adsorbents allow the formation of stable fluidized beds at high operating flow velocities.

Expanded bed adsorption is a unique technique offering advantages in recovery of proteins from crude fermentation broths. It provides increased process economy due to a decreased number of process steps, increased yield, shorter overall process

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1570-0232/02/$ – see front matter
PII: S1570-0232(02)00712-2

time [5], reduced work cost [6], and reduced running cost as well as capital expenditure [7].

Expanded bed adsorption is rapidly gaining recognition as a versatile tool for chromatography in the initial phase of downstream processing. Successful processing by EBA has been reported for myeloma cell culture [8], whole mammalian cell culture broth [9], whole hybridoma fermentation broth [10,11], and cell culture supernatant from continuous fluidized bed bioreactors [12], etc.

The potential for the use of affinity ligands in expanded bed adsorption procedures has been widely reviewed in recent work reported by Chase [13].

In the present work, the use of STREAMLINE recombinant Protein A Fast Flow chromatography for whole hybridoma fermentation broth obtained from a continuous culture using a Cell-Pharm hollow fiber bioreactor 2500 is reported. The expanded bed adsorption method is compared with conventional packed chromatography for IgG_{2b} purification.

2. Experimental

2.1. Cell line and monoclonal antibody

The hybridoma CB Hep-1 secretes monoclonal antibody (mAb) type IgG_{2b}, specific for ''a'' determinant of the recombinant hepatitis B surface antigen (rHBsAg) [14]. This mAb is used as an immunoligand in the downstream purification process of rHBsAg employed for a commercially available recombinant hepatitis B virus vaccine (Heber-Biovac HBTM, Heber Biotec, Cuba) [15]. The cells were cultured in TurboDoma HP-1 medium (Cell Culture Technologies, Zurich, Switzerland) supplemented with 1% fetal calf serum (Gibco, Grand Island, NY, USA) and 500 μ*M* ferric citrate (J.T. Baker, NJ, USA) in a hollow fiber bioreactor.

2.2. Size exclusion chromatography

A Sephadex G-25 (Amersham-Pharmacia Biotech, Uppsala, Sweden) was used as upstream step in combination with centrifugation (10 000 *g*) and filtration (0.45 μm) for the packed bed adsorption method. The filtered material (60 ml) was applied on 180 ml of Sephadex G-25 gel in phosphate-buffered saline (PBS), pH 8.0 (XK 50, Amersham Pharmacia Biotech) at a flow-rate of 130 cm/h.

2.3. Protein A chromatography

After gel chromatography, samples were applied to a protein A affinity column (Amersham Pharmacia Biotech). Two different chromatographic modes were used: packed bed and STREAMLINE modes. A 90-mg amount of harvested mAbs was loaded onto 50 ml of each column in PBS, and subsequently the columns were washed under the same conditions. The cell suspension contained 0.3 mg IgG/ml, which is in the range suggested by Amersham Pharmacia Biotech [16]. The IgG_{2b} was eluted in a single step using 0.1 *M* citric acid, pH 3.0 at flow-rates of 75 and 100 cm/h in packed bed and STREAMLINE conditions, respectively. According to the protocol outlined in Table 1 and Ref. [17] cleaning-in-place and sanitization-in-place were performed immediately after the elution step, using upward flow in the expanded bed mode at 100 cm/h. The cleaning step included a pause to increase exposure time between 4 and 15 h. The adsorbents were stored in 20% ethanol at 4 °C.

Table 1
Protocols for cleaning-in-place and sanitization-in-place

Procedure	Expanded bed adsorption	Packed bed
Cleaning-in-place and sanitization-in-place	2 *M* NaCl, 4 h Distilled water, 5×column volume 0.5 *M* NaOH/1.0 *M* NaCl during 4 h Distilled water, 5×column volume	70% ethanol, 15 h
Storage	20% ethanol	20% ethanol

2.4. Analytical assays

An enzyme-linked immunosorbent assay (ELISA) was explored to determine the content of mAb CB Hep-1 in the samples. The wells were coated with mAb CB Hep-1 and after blocking, the purified antigens were added to the wells. The plate was incubated with horseradish radish peroxidase conjugate (Sigma, St. Louis, MO, USA) and the reaction was revealed with 0.05% orthophenylenediamine and 0.015% H_2O_2 in citrate buffer (pH 5.0). To the reaction, 1.25 *M* H_2SO_4 was added. The protein concentration was determined by the Lowry test [18].

The DNA impurities were detected according to the method reported by Brown [19], whereas the protein impurities were determined by sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS–PAGE) under reducing conditions [20].

3. Results and discussion

In this study we compared STREAMLINE recombinant Protein A and packed bed Protein A chromatography. A STREAMLINE 25 was selected for method development at the laboratory scale because it gives stable expanded beds and scalable results; it allows capture of biomolecules directly from feedstocks and it also has options for a manual or hydraulic adaptor.

The profile of a culture run in a Cell-Pharm 2500 hollow fiber system demonstrated a rapid growth, saturating the extracapillary space of the bioreactor in 18 days. According to the ELISA more than 1 g of IgG_{2b} was produced during 19 days of experiments. This is in accordance with results obtained by Czirbik et al. [21]. The concentration of monoclonal antibodies increased throughout the experiment starting from 145 μg/ml and reaching 2.3 mg/ml by day 18. Details of this experiment are reported by Valdés et al. [22].

The objective of the optimization work in the expanded mode was to establish the parameter effects cells, cell debris, etc., had on expansion, binding, washing and cleaning of the matrix.

According to Table 2, the recovery rate of IgG was 1.4-fold higher in STREAMLINE recombinant Protein A Fast Flow than in PB Protein A (92 and 64%, respectively); the lower recovery obtained in the latter method seems to be caused by initial steps performed in the PB mode. On the other hand, the gel expansion could facilitate the adsorption and, as a consequence, increase the chromatographic efficiency.

The product eluted from the columns was more concentrated than the starting material in both cases, but the expanded bed method yielded sevenfold more concentrated product than the packed bed mode.

The purity measured by SDS–PAGE is illustrated in Table 2 and Fig. 1. Starting from a crude material of 30% purity, affinity chromatography yielded a highly pure final product in both expanded bed and packed bed modes and it was approximately threefold higher than the starting material.

Process time was reduced from 210 to 125 min for the EBA procedure. A shorter process time will additionally help to minimize product degradation and thus, contribute to improved product recovery.

The operation of STREAMLINE recombinant Protein A Fast Flow in the expanded mode is sometimes complicated by the presence of DNA released from hybridoma cells during the lysis procedure. Such DNA often binds to column surfaces, cell/cell debris and adsorbent beads. Released DNA may stick to the net at the column inlet where

Table 2
Purification of IgG_{2b} using STREAMLINE recombinant Protein A in a STREAMLINE 25 column*

Parameter	Expanded bed adsorption	Packed bed adsorption
Sample	300 ml cell culture	300 ml cell culture
IgG (mg)	90	90
Recovery rate (%)	92	64
Process time (min)	~125	~210
Purity of eluted material (%)	98	97

* Results represent the average of five experiments.

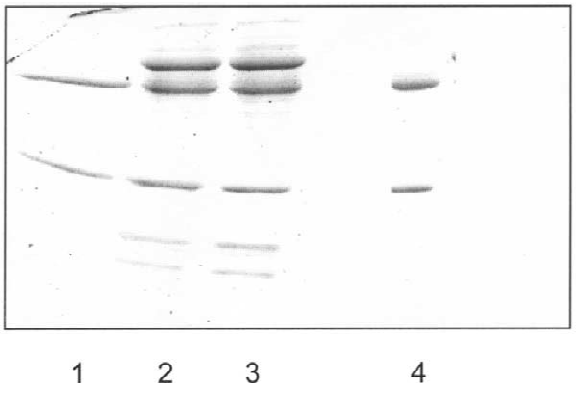

Fig. 1. Reducing SDS–PAGE of the mAb obtained from the CP2500 bioreactor and purified by packed bed Protein A and STREAMLINE recombinant Protein A affinity chromatography. Line 1: mAb control. Sample was highly purified by Protein A (99% purity, positive control). Line 2: Hollow fiber harvested material. Line 3: Product eluted from expanded bed adsorption. Line 4: Product eluted from packed bed adsorption. Samples were stained with Coomassie brilliant blue according to standard methods [20].

it can trap cells and cell debris material causing a partial blockage of the net. A blocked net will cause increasing backpressure in the system disturbing the controlled even flow necessary for a stable bed [23]

The murine DNA content is also an important variable in the final bulk; it is one of the principal drawbacks of using hybridomas for obtaining biopharmaceutical products, because of its tumorigenic characteristics. Therefore, the determination of murine DNA content in the purified samples seems to be necessary. Less than 100 pg DNA/mg mAb was found in both cases (Fig. 2). The result looks acceptable when it is compared to reports that DNA quantities of lower than 100 pg per single human dose do not cause harmful effects [24]. On the other hand, the low values of DNA indicate that the stability of the cells seems to be unaffected by the environmental conditions used in expanded bed adsorption chromatography, the low values help to conserve the adsorbent stability.

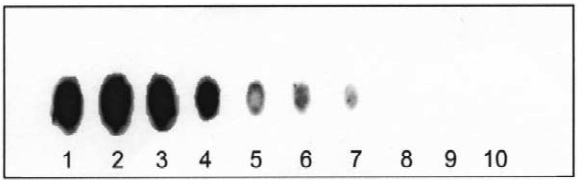

Fig. 2. Dot-blot determination of DNA contaminant levels for the mAb CB Hep-1 samples. 1–7: Standard mouse DNA material (ranging from 7.5 pg to 1 ng). 8: Negative control (100 μg of bovine serum albumin). 9: Product eluted from expanded bed adsorption. Product: Material eluted from packed bed adsorption.

Finally, the performance of the expanded bed immunosorbent was restored with NaOH as the main cleaning agent, which is a cost effective and a safe technique, since it leaves no harmful traces in the column.

4. Conclusions

This study has shown that the murine IgG_{2b} monoclonal antibodies may be efficiently purified from an unclarified hybridoma cell culture by expanded bed adsorption on STREAMLINE rProtein A in terms of higher recovery, less process time and a cleaning cost effective technique compared with the conventional packed bed method, keeping similar levels of DNA remotion and product purity.

Acknowledgements

The authors greatly acknowledge Amersham Pharmacia Biotech for supplying the samples of rProtein A Fast Flow and for their technical support.

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