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Journal of Chromatography A, 765 (1997) 201–206

JOURNAL OF
CHROMATOGRAPHY A

Purification of monoclonal antibodies by simulated moving-bed chromatography

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Received 2 July 1996; revised 9 October 1996; accepted 25 October 1996

Abstract

A simulated moving bed (SMB) system has been developed for the biospecific purification of monoclonal antibodies. Adsorption and desorption of the desired product is performed under different conditions. To increase the purity and yield of the antibodies, two purge steps have to be introduced. The steady-state performance of the SMB system was modelled by solving the governing differential equations using a linear driving force approximation. The model parameters were determined independently in batch experiments. They were used to determine the operating conditions of the SMB system for the purification of monoclonal antibodies from cell culture supernatant. The antibodies could be isolated with a yield of ≥90. SDS gel electrophoresis of the feed and product stream showed that more than 99% of the contaminating proteins were removed in a single step by SMB chromatography.

Keywords: Simulated moving bed chromatography; Monoclonal antibodies

1. Introduction

Continuous simulated moving bed (SMB) Chromatography is a powerful purification process that allows more efficient utilisation of the adsorbent capacity by maximising the driving force for mass transfer [1]. Such processes have been developed for a wide range of separations, mainly for large-scale separations of low-molecular-mass compounds in the petrochemical or food-processing industries [2]. The application for the purification of biological macromolecules has, however, received limited interest [3,4].

Affinity chromatography is a very powerful technique for the purification of biomolecules such as enzymes or antibodies and is applicable to SMB chromatography. It is a highly selective separation method, the solid phase adsorbs the desired protein specifically. Since adsorption and desorption of the desired product has to be performed under different conditions, the SMB device has to consist of at least two zones (adsorption and desorption zone).

It has been shown previously [4] that the recovery of a SMB device consisting of two zones depends on the desired product purity. Most of the impurities were eluted within the first minutes of each switching interval. A higher specific activity was achieved by wasting the extract eluted within the first min of each switching period. This resulted in a decreased product recovery. Two purge zones between adsorption/desorption steps could be introduced to increase the column yield.

The aim of this study is to investigate the design and performance of such a SMB device for the

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PII S0021-9673(96)00932-6

purification of monoclonal antibodies (MAb) by biospecific affinity chromatography.

2. Material and methods

2.1. Chemicals

The chromatographic support used for immobilization was Cyanogen bromide-activated Sepharose 4 Fast Flow from Pharmacia (Uppsala, Sweden). Recombinant Protein A was purchased from Calbiochem-Novabiochen (San Diego, CA, USA).

All immunochemicals used for the quantification of the MAbs were purchased from Boehringer (Mannheim, Germany). The specific antigen was prepared as described in Ref. [5].

All other chemicals were of analytical grade.

2.2. Monoclonal antibodies

Several monoclonal antibodies (mouse IgG1) against penicillin amidase (PA) from *Escherichia coli* (EC 3.5.1.11) have been prepared [6]. One of these was found to be suitable for the bioaffinity chromatography of PA. This antibody was chosen as the target product for the purification method described in this work. The hybridoma cell line which secretes MAbs was cultivated in a membrane-dialysis bioreactor as described in Ref. [7].

2.3. Preparation of the affinity matrix

A 7.5-g amount of the support was incubated for 3 h with Protein A solution (180 mg Protein A in 60 ml phosphate buffer pH 7.5, I=0.1 M) at room temperature. After incubation the carrier was washed and the amount of bound Protein A was determined by measuring the absorbance at 280 nm of the supernatant and the washing buffer. The ligate density was 5 mg Protein A per ml wet support.

To remove residual active groups the carrier was incubated for 2 h at room temperature with ethanolamine solution (1 M) of pH 8.0.

2.4. Determination of antibody concentration

Mouse IgG contents in the samples were determined by an antigen-specific enzyme-linked immunosorbent assay (ELISA). The concentration of pure antibodies was determined photometrically at 280 nm using a molar absorptivity of 13.5 for a 1% solution [8].

2.5. Determination of product purity

The product purity was determined by sodium dodecyl sulphate–polyacrylamide gel electrophoresis (SDS–PAGE) in a MINI-Gel System from BioRad (Richmond, CA, USA). A 10% polyacrylamide gel was prepared according to [9]. The samples were concentrated by ultrafiltration (Omegacell, Filtron, Karlstein, Germany). For protein detection a silver stain method was used (Silver Stain Kit, BioRad).

2.6. SMB apparatus

Two purge steps were added to the SMB system described previously [4]. The basic operating principle remained the same. After leaving the adsorption zone each column enters purge zone 2 in which the contaminants situated in the inter- and intraparticle volume are removed. Purge zone 1 (between the desorption and the adsorption zone) allows the equilibration of the column. Fig. 1 shows the operating principle and flow chart of the SMB system.

2.7. Determination of adsorption and desorption conditions

Protein A was immobilized in microtiter plates (Greiner, Nürtingen, Germany) and MAb solutions of different pH and ionic strength were added. The amount of Protein A not complexed with the mouse MAb was measured by reaction with sheep anti-rabbit antibodies labelled with peroxidase.

2.8. Determination of adsorption equilibrium and mass transfer coefficients

These experiments were performed with monoclonal antibodies which were purified by conventional column chromatography on Protein G Sepharose Fast Flow (Pharmacia). A suspension of the chromatographic support in 0.1 M phosphate buffer pH 7.0 was incubated for 2 h at 25°C with MAb

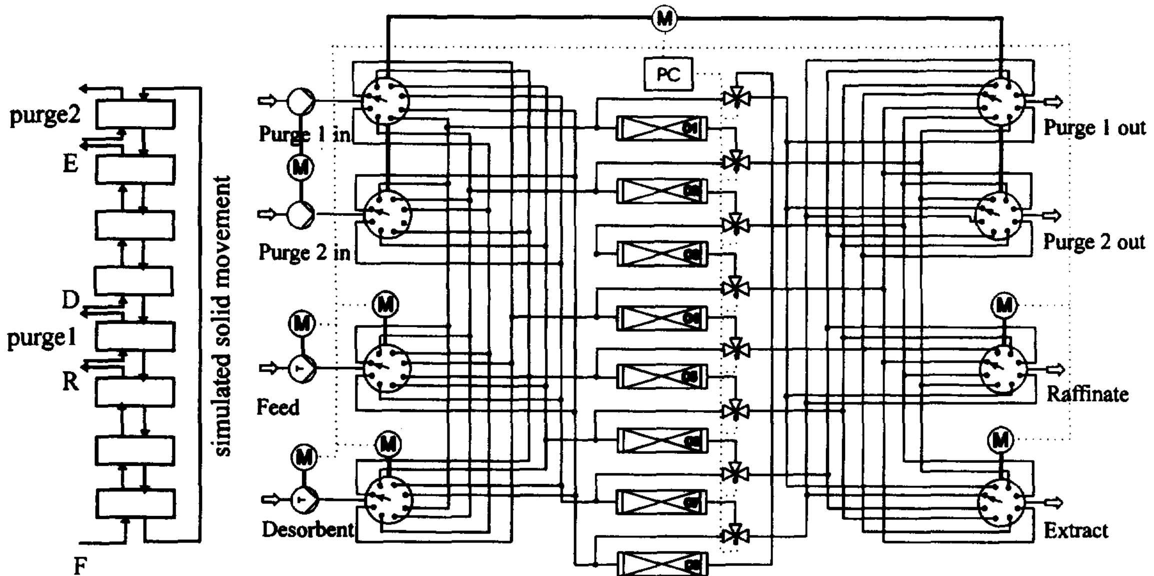

Fig. 1. Operating principle and flow chart of the SMB system. The system consists of eight columns (2 cm I.D.) filled with 1 cm height adsorbent. The solid movement is simulated by a stepwise shifting of all inlet and outlet streams by one position. This is equivalent to a movement of the columns in the opposite direction. The cell culture supernatant is introduced at point F and the desired MAb is adsorbed. The liquid leaves the SMB device as raffinate at point R. The desorbing buffer is introduced at point E and the desorbed MAb is leaving the SMB device in the extract stream (E).

solutions of different concentrations. After incubation the amount of non-adsorbed MAb was determined by measuring the absorbance at 280 nm. Mixed vessel experiments were performed to determine the rate of adsorption and desorption.

1 ml adsorbent was filled in a glass vessel and 3 ml MAb solution in 0.1 *M* phosphate buffer (pH 7.0) was added. Samples were taken to determine the amount of antibody in the fluid phase.

3. Results and discussion

3.1. Determination of adsorption and desorption conditions

The experiments showed an optimal pH for adsorption of pH 7. Desorption can be achieved by changing the pH to 3.

Fig. 2 shows the dependence of the interaction between the antibody and protein A on pH and ionic strength.

The ionic strength of the buffers used for purge and desorption determines the time necessary to shift the pH from a low value to a high value, and vice versa. To allow a short switching interval the following buffer system was used:

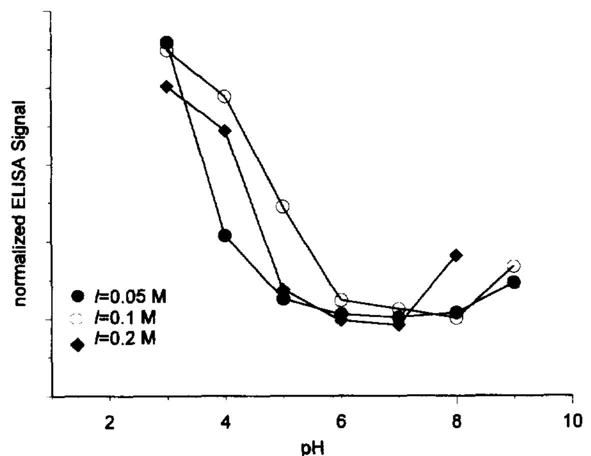

Fig. 2. Dependence of the interaction between the antibody and protein A on pH and ionic strength. The ordinate shows the relative ELISA signal of the anti-rabbit IgG bound to the Protein A binding sites not occupied by the desired MAb.

Purge 1: 200 mM phosphate buffer pH 7.0.
Desorbent: 50 mM citrate buffer pH 3.0.
Purge 2: 50 mM phosphate buffer pH 7.0.

3.2. Determination of adsorption equilibrium and mass transfer coefficients

In this concentration range the relation between the MAb concentration in the liquid phase (c^*) and in the solid phase (q^*) in equilibrium can be approximated by a linear isotherm in the form:

$$q^* = Kc^* \quad (1)$$

The distribution coefficient K was found to be 12.4.

The rate of adsorption and desorption could be calculated using a linear driving force expression:

$$\frac{dq}{dt} = k_{ad}(q^* - q) \quad \text{(for the adsorption zone)} \quad (2)$$

$$\frac{dq}{dt} = -k_{des}q \quad \text{(for the desorption zone)} \quad (3)$$

where q is the actual solid-phase concentration in $mg/ml_{adsorbent}$. The mass transfer parameters k_{ad} and k_{des} were found to be 0.002 s^{-1} and 0.01 s^{-1}, respectively.

With these values and the information about the time necessary to achieve the pH shift between the adsorption and the desorption section it is possible to choose the proper operating conditions (switching time and flow-rate) for a given feed concentration to achieve maximum yield. The loss of product in the SMB system is the sum of the loss of product by breakthrough and by purging the dead volume and the inter- and intraparticle volume of each column entering the desorption section. This can be calculated by solving the differential mass balance as described in Ref. [4] for different flow-rates of feed and solid.

Fig. 3 shows the theoretical product yield in % calculated by solving the differential mass balance of the adsorption zone.

3.3. Purification of monoclonal antibodies

Cell culture supernatant was diluted to a MAb concentration of 0.16 mg ml^{-1}. Feed and desorbent

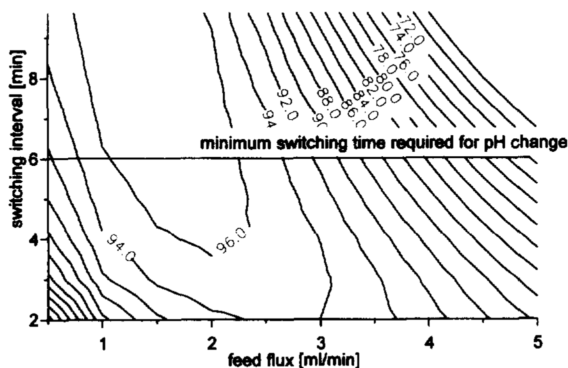

Fig. 3. Theoretical product yield in % calculated by solving the differential mass balance of the adsorption zone. The calculation takes into account the breakthrough and the loss of product by purging each column after leaving the adsorption section. The SMB consists of three columns in the adsorption section. The feed concentration is 0.16 mg ml^{-1}.

flow-rates were 0.96 and 1.25 ml min^{-1}, respectively.

To avoid irreversible denaturation of the MAb the pH of the extract was immediately adjusted to pH 7 by adding 0.2 M tris buffer of pH 9.

The steady-state concentration profile within the SMB device could be calculated by solving the differential mass balance of the desired feed component in the liquid and solid phase with the appropriate (Danckwerts) boundary conditions. The model was evaluated with a SMB system for the purification of α-chymotrypsin by affinity chromatography as described previously [4]. Although the linear driving force assumption is a rather rough approximation the calculated concentration profile showed good agreement with the concentration profile determined experimentally.

Fig. 4 shows the concentration profile within the SMB device at steady state.

The MAb could be purified with a yield of active antibody of 90%. The MAb concentration found in the extract stream was 70% of the feed concentration. Not all the product was eluted in the desorption zone. About 2% of the feed was found in purge zone 1. This fraction was pooled with the extract. This effect was also observed in purification experiments with a single column under the same conditions. Hence, this effect is due to the change of

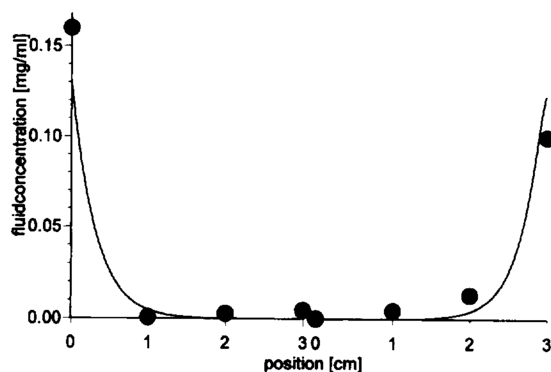

Fig. 4. Concentration profile within the SMB device at steady state; (●) measured MAb concentration (ELISA), (–) calculated concentration profile.

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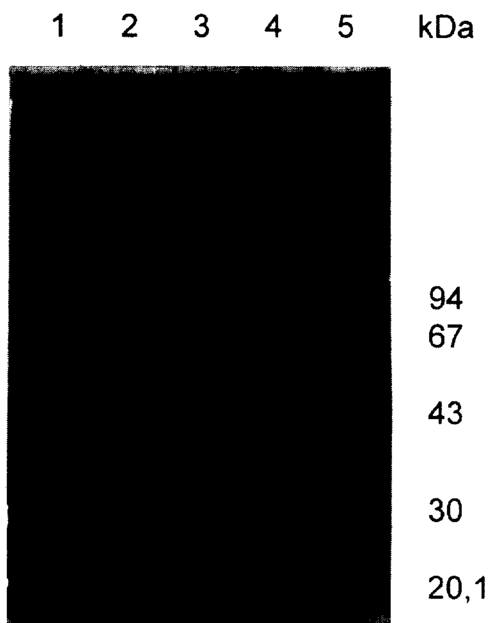

Fig. 5. SDS–PAGE (silver stain) of feed and extract. Lanes: 1, feed (concentrated 10-fold by ultrafiltration); 2, extract (concentrated 10-fold); 3, marker proteins; 4, feed; 5, raffinate (concentrated 10-fold). The SDS–PAGE of the concentrated extract stream shows two MAb bands (light and heavy chain). Without concentrating the extract sample the protein band at 30 kDa is not detectable, indicating a product purity of >99%.

ionic strength in the desorption and equilibration step and is not due to the SMB operation.

Fig. 5 shows the SDS–PAGE profile of feed and extract.

3.4. Comparison between SMB chromatography and conventional column chromatography

One advantage of the SMB technology is the continuity of the process. To apply a continuous purification scheme with discrete columns one has to use at least 4 columns, each one operating in either the adsorption, purge, desorption or regeneration mode. This can be done either by using 4 columns with lengths corresponding to the length of the SMB adsorption zone, which would increase the amount of solid, or by splitting the same amount of solid into four columns.

In this case the adsorption step has to be stopped after 3 min to get the same yield as in the SMB experiments. Because of the lower loading of the solid phase this results in a greater dilution of the product (only 10% of the feed concentration could be obtained under the same conditions used in the SMB experiments). Although this could be optimised, this result shows clearly the advantage of a SMB purification scheme.

4. Conclusions

The recovery of a two-section SMB device depends on the desired extract purity. By adding two purge steps to the SMB system monoclonal antibodies could be isolated directly from cell culture supernatant with a yield of ≥90%.

Based on a linear driving force approximation the predicted concentration profiles showed good agreement with the experimental results. Hence, this simple model is a good tool for the design of a SMB system applied to biospecific affinity chromatography. The model parameters can be determined independently in batch experiments.

In contrast to conventional column chromatography simulated moving-bed chromatography offers not only the advantage of a continuous process but also higher utilisation of the adsorption capacity of the solid phase, and, therefore, a smaller dilution of

the product. This method is not only applicable to the purification of monoclonal antibodies but also for polyclonal antibodies. This is because the interaction with Protein A used in this work occurs in the constant region of the antibodies.

Acknowledgments

This work was partly supported by the Graduiertenkolleg Biotechnologie funded by DFG (Ka 505/5-6).

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