Experimental and simulation analysis of membrane adsorbers used for the primary capture step in antibody manufacturing

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Membrane chromatography is a novel protein purification technique developed to overcome the major limitations due to packed beads, such as long process time, mass transport controlled by diffusion and high pressure drops.
In this work, the adsorption of human IgG onto new Protein A affinity membranes has been studied in detail. To this aim, several chromatographic cycles have been measured at different experimental conditions. A mathematical model for protein purification with affinity membrane adsorbers has been developed and validated with experimental data.

1. Introduction

Protein A chromatography has become the preferred choice for the capture step of antibody manufacturing. However, its high costs and the growth of antibody applications has driven the search for alternative technologies (Low et al. 2007). Membrane affinity chromatography is one of the processes that are receiving increasing attention as a possible alternative to bead-based chromatography, even if its industrial application is still far afield due to the low binding capacity of affinity membranes with respect to chromatography beads (Thommes and Etzel 2007).
In this work, a new affinity membrane endowed with a very interesting binding capacity for human IgG is studied in view of its use in the capturing step of a monoclonal antibody production process. The membranes have been extensively tested with pure polyclonal IgG solutions and with a cell culture supernatant containing IgG_1. The effects of flow rate and IgG concentration in the feed on the separation performance have been studied in detail, considering binding capacity, selectivity and process yield.
A model simulation of the complete affinity cycle has been developed and the experimental results have been compared with the simulated behaviour for different values of feed concentrations and feed flow rates.

2. Experimental

2.1 Materials and methods

A new Protein A membrane (Sartorius Stedim Biotech, GmbH), with nominal pore size of 0.45 μm, an average thickness of 200 μm and a void fraction of 55%, has been used for chromatographic separation of IgG. Pure polyclonal IgG, Gammanorm

(Octapharma, Sweden) and a cell culture supernatant containing IgG_1 (ExcellGene, Switzerland) were used as feed solutions.
The measure of IgG concentration of pure solutions was determined by UV readings at 280 nm (Shimadzu UV-1601). For complex solutions IgG concentration was determined by HPLC using a protein A affinity cartridge (PA ID, Applied Biosystems, Monza, MI, Italy) mounted on a liquid chromatography system (Alliance 2695 equipped with a dual wavelength UV detector 2487, Waters Milano, Italy). The purity of the eluted fractions was analyzed with size exclusion HPLC using a SEC column (Proteema 300, PSS, Mainz, Germany).

2.2 Dynamic experiments
Complete chromatographic cycles, adsorption, washing and elution have been performed using an Akta Purifier chromatographic system (GE Healthcare, Italy). A membrane column was prepared by cutting membrane discs of 2.5 cm diameter and inserting several membrane layers in a membrane holder; experiments have been carried out using layered stacks of 5 and 10 membranes.
Phosphate buffered saline, PBS, pH 7.4 was used as equilibration and washing buffer, 0.1 M glycine pH 3.5 as elution buffer and a solution of 1 M NaCl and 50 mM NaOH was used for regeneration. In experiments with pure IgG solutions, regeneration was performed every 5^{th} cycle while in experiments with the cell culture supernatant it was carried out after every cycle. The effects of different operating conditions, in particular flow rate and IgG feed concentration, on membrane performance have been thoroughly investigated.

2.3 Results
The membranes have been initially characterized in batch experiments with pure IgG solutions in order to obtain kinetic and equilibrium thermodynamic parameters. In particular, the Langmuir dissociation constant and the maximum static binding capacity have been obtained in previous work, as reported by Boi et al. 2007a, and are around 0.0934 mg/ml and 12.75 mg/ml, respectively.
Dynamic experiments with pure IgG solutions have been performed at different values of the feed concentration in the range of 0.15÷2.0 mg/ml and at flow rates ranging from 1 ml/min to 10 ml/min. The effect of concentration has been investigated in experiments performed at a constant flow rate in all process stages. As the concentration increases the breakthrough curve is sharper, indicating a better membrane utilization, and the onset of breakthrough is anticipated as can be observed in Fig. 1. Conversely, the effect of flow rate at a constant feed concentration is quite modest on the dynamic binding capacity.

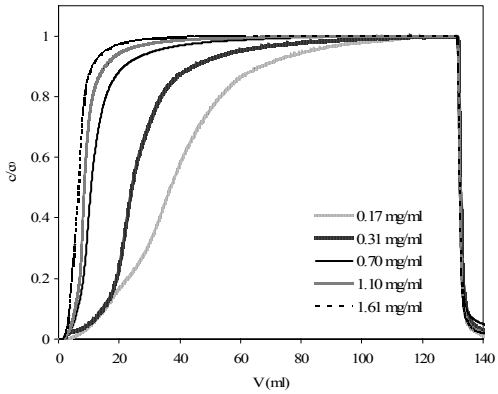

Fig. 1. Dimensionless adsorption and washing profiles at 10 ml/min for different values of IgG concentration in the feed.

Elution profiles for the same value of IgG concentration in the feed are shown in Fig. 2. As observed in previous works (Briefs and Kula, 1992, Dimartino et al. 2007) elution peaks are higher and narrower at low values of the feed flow rate and became shorter and broader as the flow rate increases.

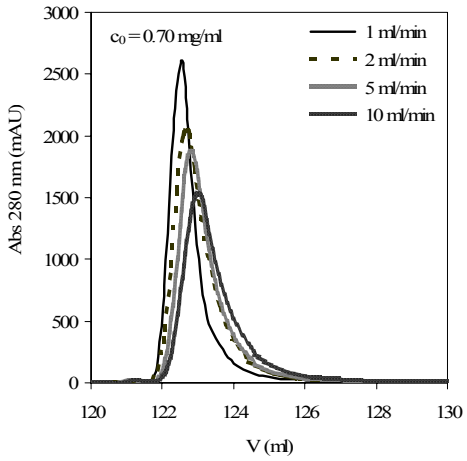

Fig. 2. Effect of flow rate on elution performance for experiments performed with 0.70 mg/ml of IgG in the feed and a layered stack of 10 membranes.

Due to the low titer in IgG, around 0.1 mg/ml, the cell culture supernatant was used as received. Experiments were performed at different flow rates and a typical absorbance profile is shown in Fig. 3. Fractions have been collected every millilitre and analyzed with both Protein A and SEC HPLC analysis. The results indicate that the elution peak is formed by pure IgG and that the membranes are endowed with very good selectivity.

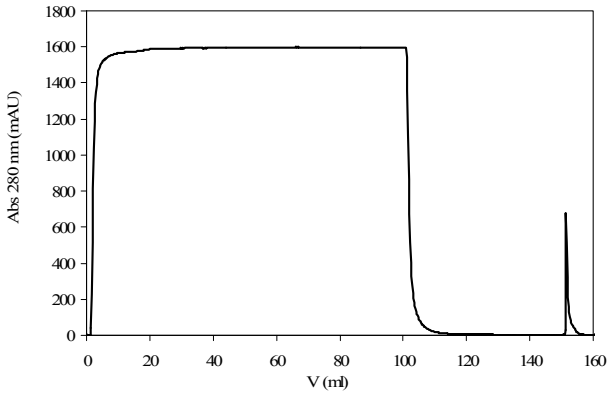

Fig. 3. Absorbance profile for experiments performed with the cell culture supernatant at 5 ml/min constant flow rate using a stack of 5 membranes.

3. Mathematical model

3.1 Theoretical basis and model solution

The model is based on the adsorption of a single protein on affinity membranes and is able to describe all process stages, namely adsorption, washing and elution. The mathematical description considers the effects of system non idealities, generally called system dispersion, due to mixing, channelling, dead-volumes, that are particularly important in bench scale membrane chromatography (Sarfert and Etzel 1997).

The mathematical formalism adopted consists in a mass balance over the membrane column coupled with a kinetic equation that describes the interactions between the protein and the ligand immobilized on the membrane matrix. Description of the adsorption and washing stages is based on the model developed by Suen and Etzel 1992, while the elution step is described with a first order kinetic equation.

The model equations, together with the relevant initial and boundary conditions, have been implemented in Aspen Custom Modeler© simulation environment and solved using the finite difference method. In industrial applications, especially for antibody capture, chromatographic columns are operated up to 10% breakthrough. Since the onset of breakthrough is the most important part of the curve, fitting of the adsorption stage has been carried out up to 50% breakthrough.

The model has been validated using experimental data obtained for the adsorption of pure polyclonal IgG on affinity membranes under different operating conditions (Dimartino et al. 2007, Boi et al. 2007a).

3.2 Simulation results

The adsorption and washing profiles calculated with the model considered have been compared with experimental results obtaining a rather satisfactory agreement with the data, as it can be noted from Fig. 4.

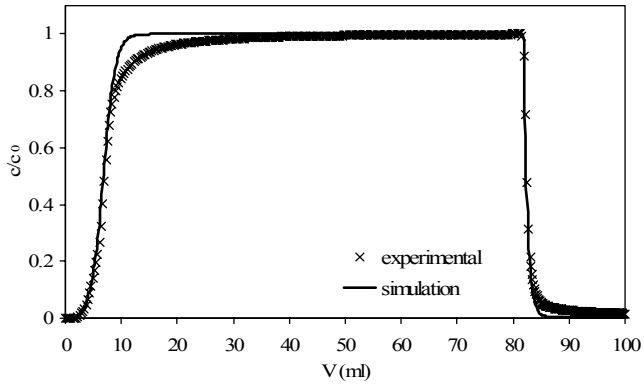

Fig. 4. Comparison between experimental data and model results for experiments performed with pure IgG solution at 1.48 mg/ml in the feed and constant flow rate of 10 ml/min using a layered stack of 10 membranes.

The model is able to describe well all process stages, as it can be observed in Fig. 5 in which the IgG profile, obtained by Protein A analysis, has been compared with the simulation results, for the experiments with the cell culture supernatant.

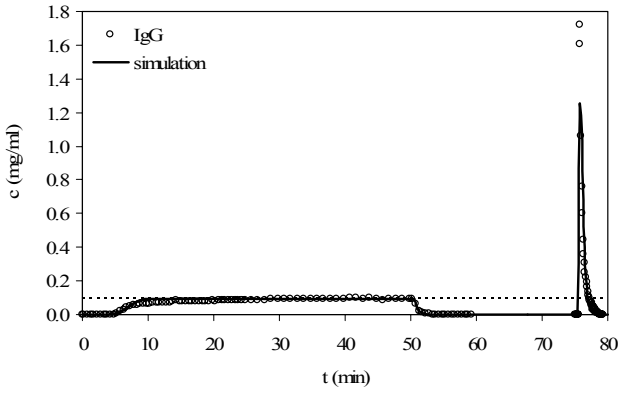

Fig. 5. Comparison between experimental results and simulations for IgG purification from the cell culture supernatant at a flow rate of 2 ml/min using a layered stack of 5 membranes.

4. Conclusions

A new protein A affinity membrane has been experimentally characterized to determine its potential application in an IgG capture step of an antibody manufacturing process. The results obtained indicate that this new membrane is endowed with a high binding capacity and very good selectivity for IgG, thus they can be used to overcome the throughput limitation and other well known drawbacks of traditional bead-based chromatographic columns.

A mathematical model that describes membrane affinity chromatography has been developed and validated considering the system under investigation (protein A affinity membrane-human IgG). This model satisfactorily describes all the process stages and is an useful tool for process design and for the simulation of a capturing step based on membrane adsorbers.

Acknowledgement

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