ANALYSIS OF DIFFERENT AFFINITY MEMBRANES FOR THE PRIMARY CAPTURE STEP IN ANTIBODY MANUFACTURINGS

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Introduction

Protein A affinity chromatography is the most widespread method for the primary capture step in antibody manufacturing [1,2] . Due to the high cost of this natural ligand, produced with recombinant *Staphyloccucus aureus*, the risk of leaching and the low stability in sodium hydroxide, that is commonly used as sanitizing agent, the interest towards new synthetic ligands is of increasing importance [3].

In this work, new affinity membranes prepared by immobilizing two different protein A mimetic ligands are considered for 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 parameters like binding capacity, selectivity and process yield have been studied in detail.

The behavior and efficiency of the different affinity membranes will be presented and will be also compared with data available for a commercially available Protein A affinity membrane.

Materials and methods

Affinity membranes

Sartobind epoxy membranes (Sartorius Stedim Biotech GmbH, Göettingen, Germany) are stabilized reinforced cellulose membranes with active epoxy groups for ligand coupling; these membranes have a nominal pore size of 0.45 μm, an average thickness of 275 μm and a void fraction of 0.68.

Two different ligands have been immobilized on the membrane supports: A2P and the new B14, both ligands, designed to bind the Fc region of the antibody, are alternatives to protein A produced by Prometic Biosciences Ltd (Cambridge, UK). A2P was designed for the capture of polyclonal antibodies, whilst B14 for the capture of monoclonal antibodies. These synthetic ligands are stable in sodium hydroxide up to 1 M concentration, as a consequence they are easy to clean and sanitize.

Affinity membranes have been prepared by the Analytical Chemistry group at the University of Vienna by immobilizing A2P and B14 ligands on the Sartobind epoxy membranes generating an optimized spacer arm, thus obtaining A2P-TA-Sartoepoxy and B14-TA-Sartoepoxy, respectively

Sartobind Protein A® (Sartorius Stedim Biotech GmbH, Göettingen, Germany) was used as a benchmark affinity membrane.

Feed solutions

A human polyclonal antibody, namely Gammanorm (Octapharma, Stockholm, Sweden), was used as a pure IgG feed solution. A cell culture supernatant (Excell Gene, Switzerland) containing human IgG_1 at a concentration of about 0.12 mg/ml was used as a source of monoclonal antibody.

Phosphate Buffered Saline, PBS pH 7.4, was used as equilibration and washing buffer; 0.1 M glycine pH 2.8, 0.05 M citric acid pH 2.5 and 0.05 M acetic acid were used as elution buffers. Regeneration was accomplished using a solution of NaOH 0.6 M.

Experimental methods

Batch experiments have been performed by soaking the membranes in a IgG solution kept in gentle agitation using an orbital shaker until equilibrium was reached. The protein concentration was measured at the beginning and at the end of each experiment and the amount of protein adsorbed was calculated with a material balance.

Dynamic experiments have been performed using an FPLC AKTA Purifier 100 controlled by Unicorn software (GE Healthcare, Milan, Italy). In both experiments the membranes were cut into circles of 2.5 cm in diameter; for the dynamic runs a layered stack of membranes was housed in a flow cell.

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).

Results

Batch experiments

Equilibrium adsorption experimental data, obtained in a batch system, were fitted using the Langmuir model and the relevant thermodynamic parameters are reported in table 1. The new affinity membranes are endowed with a higher binding capacity than the benchmark Sartobind Protein A membrane, as it can be observed in Fig. 1, in which the experimental data for the three membranes are reported together with the relevant Langmuir isotherms.

Table 1. Thermodynamic equilibrium parameters of the different membranes considered

Affinity Membrane	q_{max}		K_d
	($\mu g/cm^2$)	(mg/ml)	(mg/ml)
Sartobind Protein A	56.2	2.04	0.278
A2P-TA-Sartoepoxy	96.2	3.24	0.0444
B14-TA-Sartoepoxy	71.8	2.61	0.0540

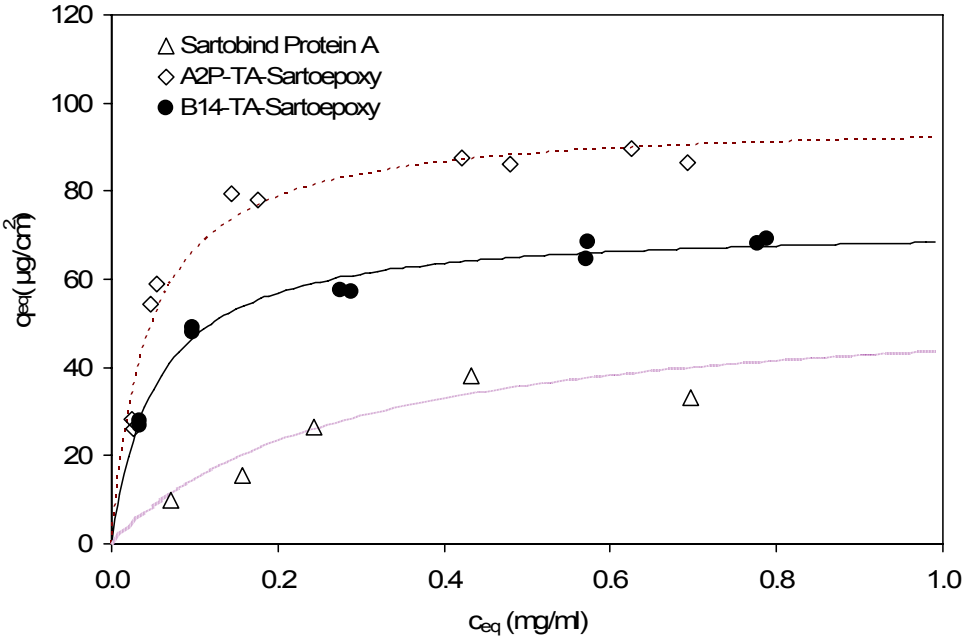

Figure 1. IgG equilibrium adsorption isotherms of three different affinity membranes

The recovery of the IgG adsorbed was very difficult from A2P-TA-Sartoepoxy membranes which are endowed with the highest binding capacity. Among the different elution buffers tested, acetic acid gave better performance, but only 59% of the IgG adsorbed was recovered. In Fig. 2 the amount of IgG eluted, using glycine and acetic acid, is reported versus the amount of IgG adsorbed.

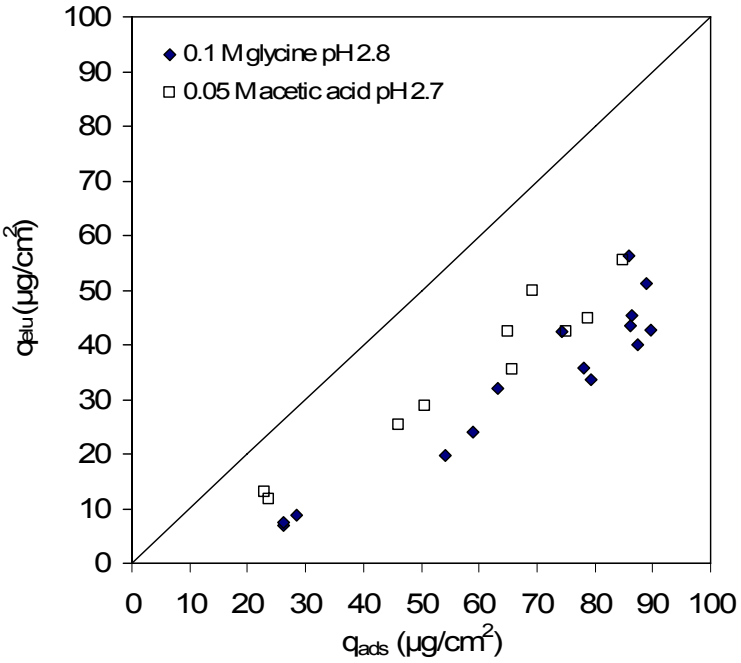

Fig. 2. Elution performance of A2P-TA-Sartoepoxy affinity membranes.

The strong binding between IgG and A2P ligand, has been already observed in previous work using different membranes [4] and is an important factor to consider in view of a possible industrial application of these membranes.

This behavior was not observed with membranes prepared with the new B14 ligand, for which good recoveries were obtained with all elution buffers, even if glycine, in this case, gave the best performance, with an average recovery of 92 %, as it can be observed in Fig. 3.

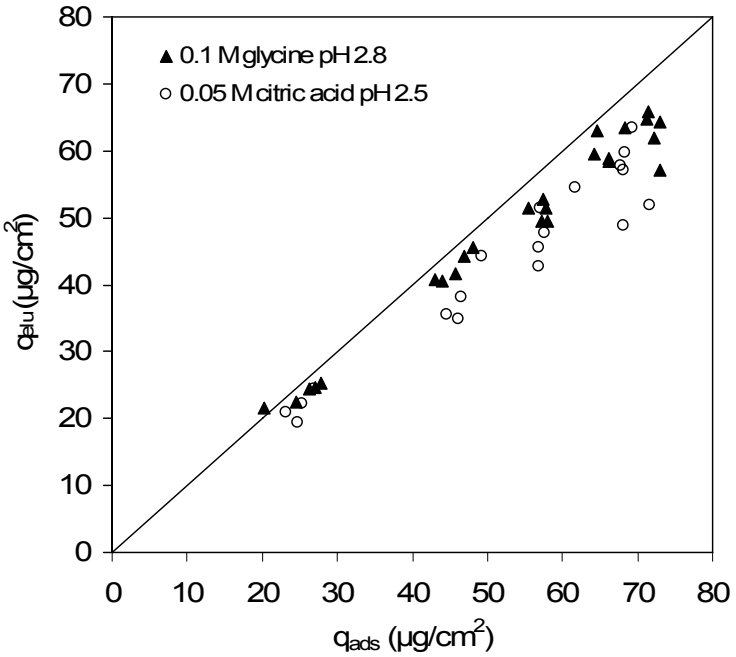

Fig. 3. Elution performance of B14-TA-Sartoepoxy affinity membranes.

Dynamic experiments

The affinity membranes were tested in dynamic experiments using pure IgG solutions and a cell culture supernatant which contains monoclonal IgG_1. With pure IgG solutions several runs were performed to study the effect of feed concentration and flow rate on membrane performance.

A comparison between A2P-TA-Sartoepoxy and B14-TA-Sartoepoxy membranes confirmed the results obtained in batch experiments, with A2P-TA-Sartoepoxy membranes endowed with higher dynamic binding capacity and lower recoveries, as it can be seen from the data reported in table 2.

Table 2. Results obtained in experiments with pure IgG at a feed concentration of 0.8 mg/ml

Affinity Membrane	$DBC_{100\%\ BT}$		Recovery
	(µg/cm²)	(mg/ml)	(%)
A2P-TA-Sartoepoxy	70.8	2.57	57
B14-TA-Sartoepoxy	60.1	2.18	93

In experiments with the cell culture supernatant fractions were collected every milliliter and analyzed with both Protein A and SEC HPLC; in addition the most relevant fractions were analyzed also with SDS-PAGE gel electrophoresis under denaturing conditions. A typical run at a flow rate of 5 ml/min for a stack of five B14-TA-Sartoepoxy membranes is shown in Fig. 4.

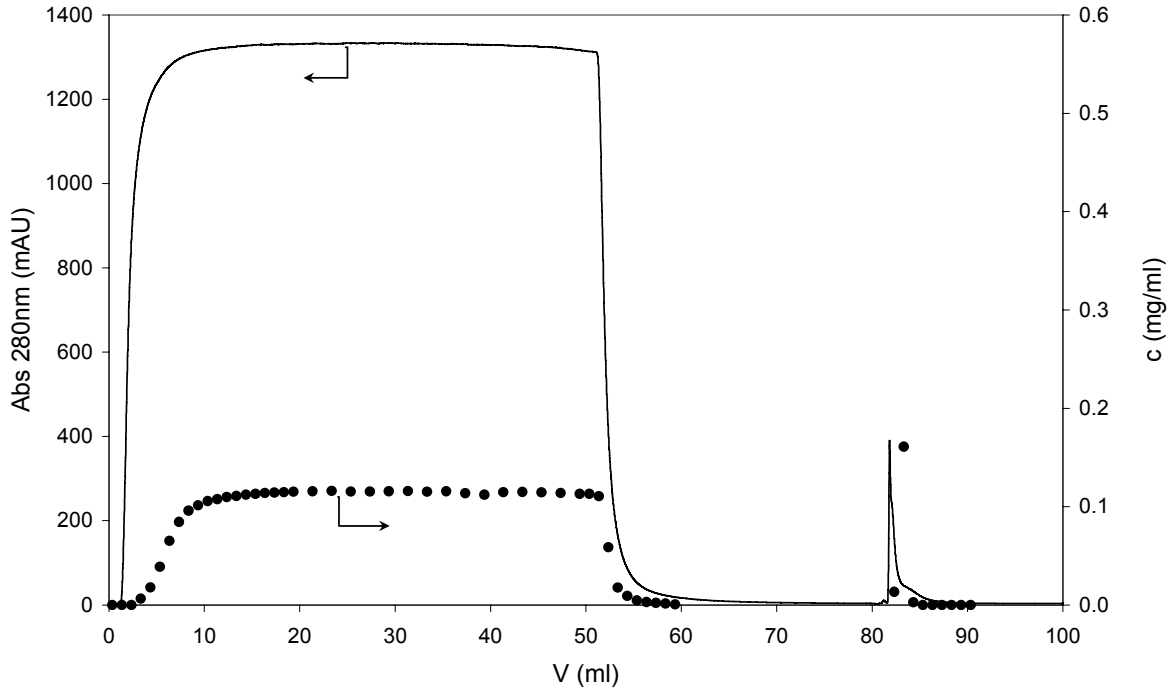

Fig. 4. Absorbance and IgG concentration profiles for experiments performed using the cell culture supernatant at a flow rate of 5 ml/min.

A direct comparison between the two membranes can be done by observing the SDS-PAGE electrophoresis that was run using fractions collected at the same volume for both membranes (Fig. 5). From the eluted fractions labeled E2, it can be observed that A2P-TA-Sartoepoxy membranes are not selective for IgG where IgG heavy chains can be barely detected, while for B14-TA-Sartoepoxy membranes both IgG heavy and light chains can be easily identified.

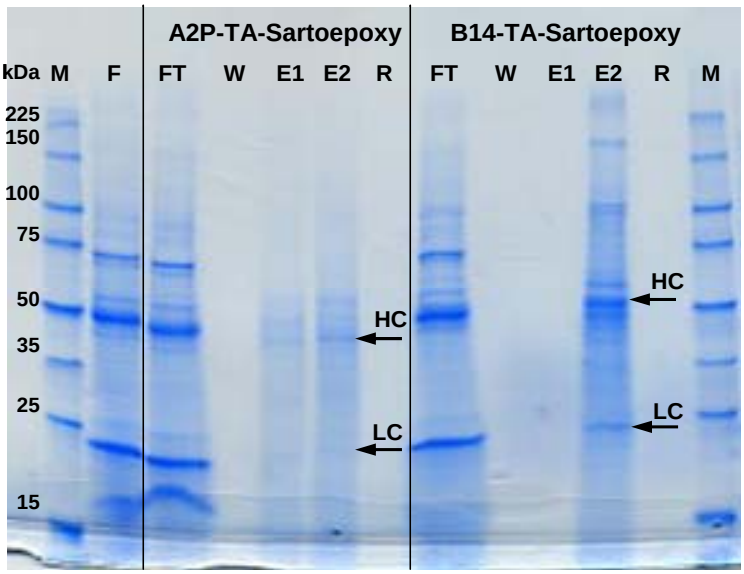

Fig. 5. SDS-PAGE electrophoresis under reducing conditions of the fractions collected during dynamic experiments performed with the cell culture supernatant. M: molecular weight markers; F: feed; FT: flow through; W: washing; E1 and E2: elution fractions; R: regeneration. HC labels IgG heavy chains and LC labels IgG light chains.

Conclusions

New affinity membranes have been experimentally characterized to determine their potential application in an IgG capture step of an antibody manufacturing process. The results obtained indicate that these new membranes are endowed with a good binding capacity for IgG, higher than the capacity of the commercially available Sartobind Protein A affinity membranes.

Although A2P-TA-Sartoepoxy membranes possess the highest binding capacity, B14-TA-Sartoepoxy membranes are superior in terms of IgG recovery and selectivity, thus they are worth further investigation to better evaluate their possible industrial application.

Acknowledgement

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References

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