Capture of mouse monoclonal antibodies

by cation exchange chromatography

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Capture of mouse monoclonal antibodies from cell culture supernatant by cation exchange chromatography

Abstract

Monoclonal mouse IgG1 antibodies, dedicated to the preparation of an immuno-affinity chromatography matrix, have been efficiently captured from a cell free culture supernatant by binding to high capacity tentacle-type strong cation exchangers (Fractogel® EMD SO_3^- (M) or Fractogel® EMD SE Hicap (M)). These chromatography resins displayed the highest static as well dynamic protein binding capacities among a series of examined materials, regardless of the process conditions (pH, conductivity, linear flow rate). Even at pH 6.5, in 30 mM sodium phosphate buffer with 30 mM sodium chloride, more than 19 mg of IgGs could be bound per ml of packed resin at 220 cm/hr and with a yield of more than 95 %. Flow rates up to 400 cm/hr were also successfully used.

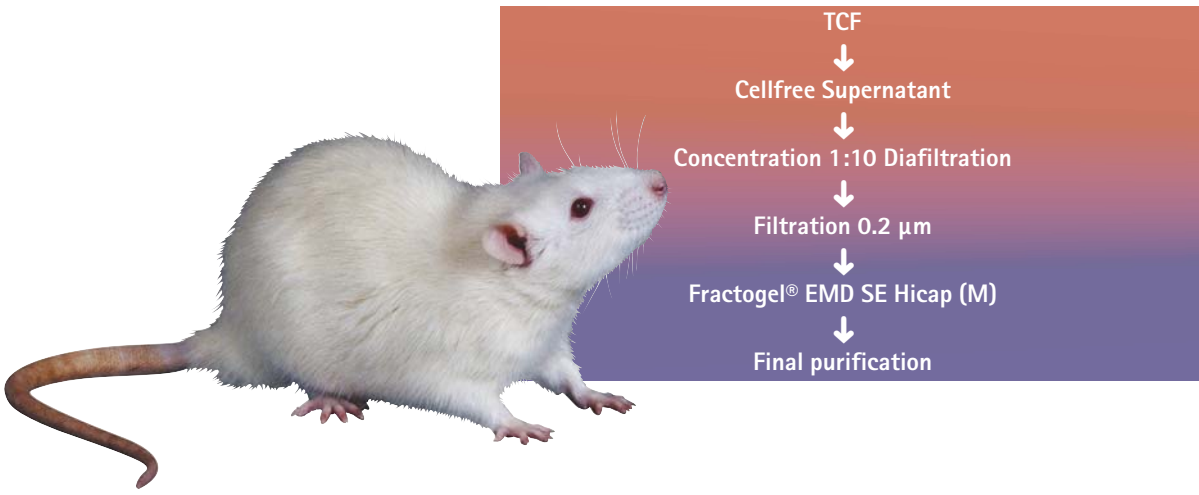
In a number of validation experiments each time approximately 4 g of antibodies from 40 l fermentation broth were chromatographed on a 5.0 x 16.8 cm column (c.v. 330 ml) at 40 - 80 ml/min using CIP with NaOH/NaCl after each run. The resulting antibody preparation was more than 90 % pure after this step, according to SDS-PAGE.

In addition, the protein capturing by the cation exchanger was superior to an earlier used PEG precipitation step, because it is faster and can be performed in a closed system. Furthermore, it is intended to improve the removal of nucleic acids and endotoxins from the sample by the ion exchange chromatography step.

Results

A purification protocol for monoclonal mouse IgG1 antibodies was developed which takes advantage of the extraordinary high protein binding capacity of new tentacle-type cation exchange chromatography supports. These cation exchangers allow to capture the desired protein efficiently from filtrated TCF even in the presence of 30 mM sodium chloride and at pH 6.5, conditions which are necessary on one hand to maintain the stability of the sample protein solution, but which on the other hand do not perfectly match the prerequisites of cation exchange processes. The respective conditions mainly prevent HSA – a content of the cell culture medium – from precipitation which, if it occurs, gives rise to co-precipitation of IgG and concomitant loss of yield.

Fig 1:
Overview on the general purification scheme for affinity antibodies from tissue culture filtrate.



The salt content also provides a "salting-in" for the proteins in the sample.

Fig. 1 shows the general downstream processing protocol used for the antibody production. Cation exchange chromatography was considered as a useful protein capturing step, because it allows at the same time to separate the imunoglobuline fraction from cell culture medium ingredients like HSA and Phenol Red. Furthermore, this chromatography step contributes to the removal of DNA and endotoxins from the culture filtrate.

A survey of suitable available cation exchanger resins was carried out, in order to find a material which is capable to bind IgG in sufficient amount per ml of gel and which gives a high yield in purified product. A number of gels were able to bind the sample IgG under the above mentioned conditions. The amount of bound IgG per ml of gel clearly depended on the amount of sample load and were found to be at the highest level when tentacle-type strong cation exchangers (Fractogel® EMD SO_3^- (M) or Fractogel® EMD SE Hicap (M)) were used (Fig. 2). The property of the Fractogels persisted when the comparison was carried out under varied conditions, e.g. for different buffer concentrations in the column feed stream as is shown in Fig. 3 for two of the gels with high binding capacity.

Further optimization of the cation exchange step showed, that Fractogel® EMD SE Hicap (M) could bind approximately 18 of applied 20 mg of IgG in the presence of 30 mM salt and 30 mM phosphate buffer at pH 6.5 (Fig. 4), conditions which proved to give satisfactory results with respect to recovery of protein and biological activity of the purified product. In a long term study with repeated chromatography cycles, performed with different levels of sample load and using integrated CIP with 1 M sodium hydroxide after each individual run, the new resin proved to be highly stable. Almost no changes of the column parameters of chromatographic results could be observed (Fig. 5).

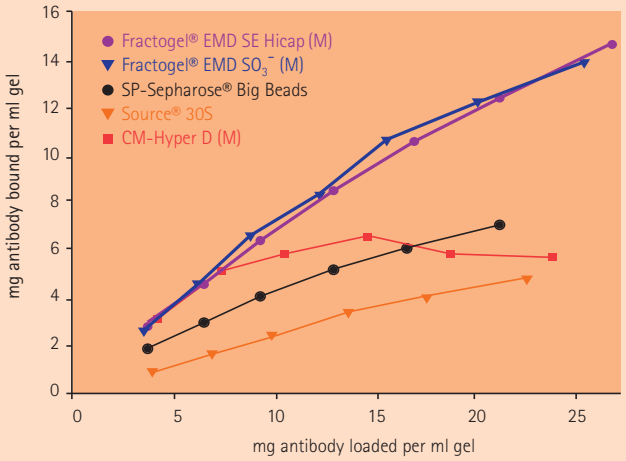
Using optimized conditions for the cation exchanger a number of validation experiments were performed, each time loading approximately 4 g of antibodies from 40 l fermentation broth onto a 5.0 x 16.8 cm column (c.v. 330 ml) at 40 - 80 ml/min including CIP with 1 M NaOH/NaCl after each run. A typical chromatogram is shown in Fig. 6. The total recovery of IgG was around 80 % this step or about 88 % for the ion exchange step alone (Tab. 1). The resulting antibody preparation was more than 90 % pure after this step, according to SDS-PAGE (Fig. 7). By slightly increasing the column bed volume the overall recovery could be increased to over 90 %. It should also be mentioned that the product eluted from the cation exchanger can be used directly for the following Protein A affinity chromatography step.

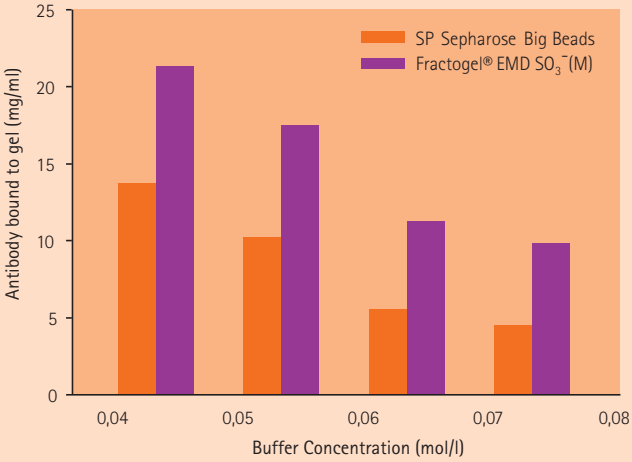
Fig 2:
Binding of diafiltrated antibody concentrate onto different cation exchange gels.
Buffer A: *0.03 M sodium phosphate, 0.03 M NaCl, pH 6.5*
Buffer B: *0.03 M sodium phosphate, 1 M NaCl, pH 6.5*
Linear flow rate: 400 cm/hr

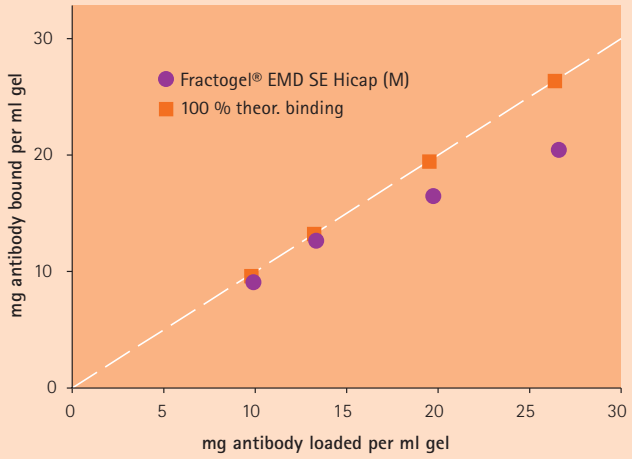
Fig 3:
Binding of diafiltrated antibody concentrate at pH 6.5 onto SP-Sepharose® Big Beads and Fractogel® EMD SO_3^- (M) at different buffer concentrations. The buffer concentration is expressed as the sum of the molarities of sodium chloride and sodium phosphate.

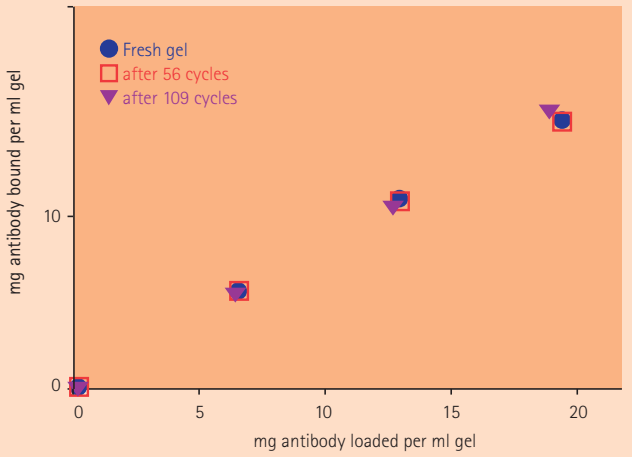
Fig 4:
Binding of affinity antibody onto Fractogel®EMD SE Hicap (M) after optimization. The dashed line represents the theoretical values for quantitative binding of the protein.
Column: *1.0 x 3.5 cm (c.v. = 2.75 ml gel)*
Linear flow rate: 400 cm/hr

Fig 5:
Stability of Fractogel®EMD SE Hicap (M) to treatment with 1 M sodium hydroxide. The long term stability of the gel against repeated cleaning-in-place (CIP) cycles was examined by measuring the antibody binding capacity at different levels of protein loading in a packed column after the individual cycles. Each alkaline cleaning step was performed with eluent C for 1 h.
Column: *1.0 x 3.8 cm (c.v. = 2.98 ml gel)*
Flow rate: *3 ml/min*
Eluent A: *0.03 M Na-phosphate, 0.03 M NaCl, pH 6.5*
Eluent B: *0.03 M Na-phosphate, 1 M NaCl, pH 6.5*
Buffer C: *1 M NaOH*









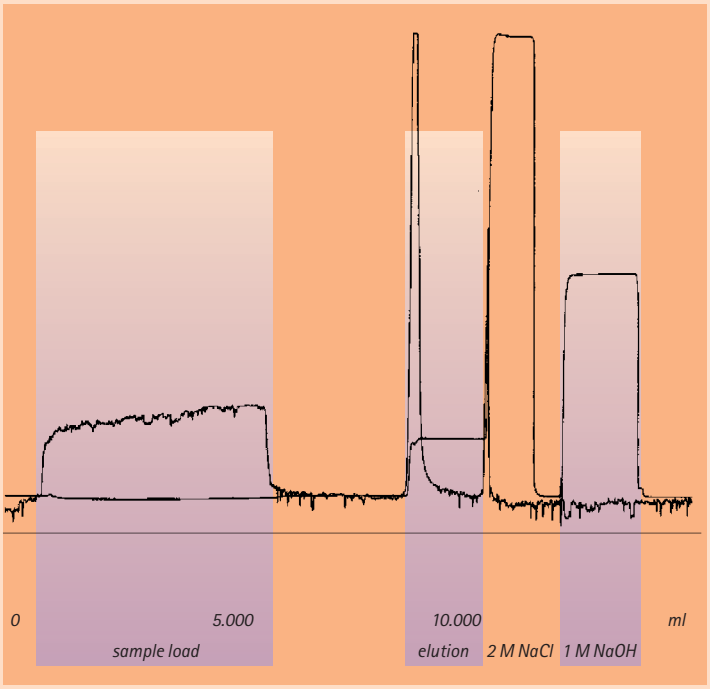


Fig 6:

Sample chromatogram for the capturing of monoclonal affinity antibody on a column packed with Fractogel® EMD SE Hicap (M).

Column: 5.0 x 16.8 cm (c.v. = 330 ml)

Sample: 4.6 g antibodies from 40 l fermentation broth

Flow rate: 60 ml/min

The breakthrough contained 0.36 g of protein, whereas 3.97 g were eluted in the step gradient. The NaCl wash following the elution step contained less than 1% of the total protein load. With the NaOH wash only traces of residual material were eluted from the column.

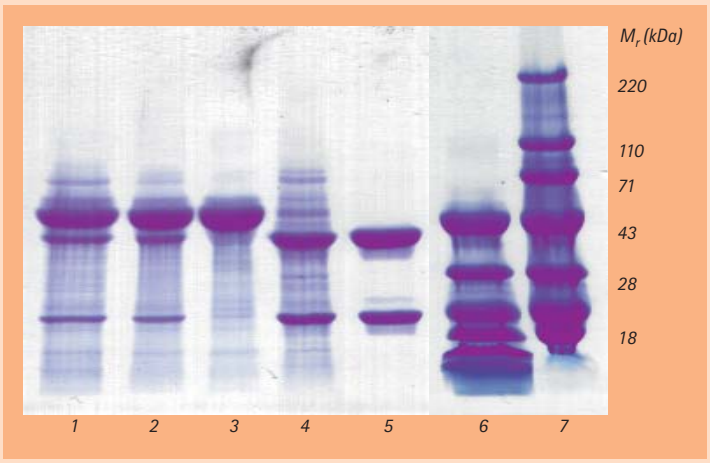


Fig 7:

SDS-Page of samples taken from the individual steps of affinity antibody purification.

1 - Tissue Culture Filtrate (TCF)
2 - TCF after Diafiltration
3 - Flowthrough HiCap
4 - Elution HiCap
5 - Purified Antibody
6 - Prestained Marker Low
7 - Prestained Marker High

Tab. 1: *Overview of antibody purification*

	Total Protein (1)		Total IgG (2)		IgG/Protein Ratio
Step	(g)	yield (%)	(g)	yield (%)	
TCF	29.30	100	4.71	100	0.16
Cellfree diafiltrated Concentrate	25.90	88	4.30	91	0.17
Eluate after Cation-Exchange Chromatography	5.09	17	3.77	80	0.74

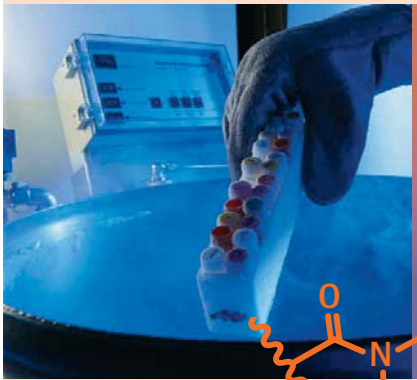
(1) Average of 3 Experiments
(2) Average of 5 Experiments

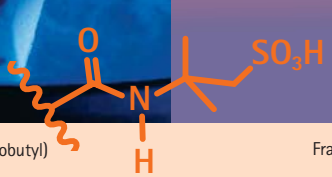
Discussion

The capturing of monoclonal antibodies from cell culture filtrates by cation exchange chromatography usually suffers from low efficiency, because the conditions necessary to avoid protein precipitation prevent IgG from complete binding to the respective chromatography support. At pH 6.5 and in the presence of 30 mM buffer and 30 mM salt the loss of product by the precipitation of serum albumin and the co-precipitation of immunoglobulin no longer occurs. However, most of the known strong cation exchanger supports have very low binding capacity for IgG under these conditions. On the other hand, cation exchange is among the best strategies for the direct capturing, because at the same time it allows not only to separate the antibodies efficiently from the bulk of serum albumin, but also to get rid of nucleic acids and the Phenol Red which are present in the crude preparation. These components are found in the breakthrough of the cation exchanger column. Furthermore, this step represents an important contribution to get rid of viruses and endotoxins which may also be present in the crude material.

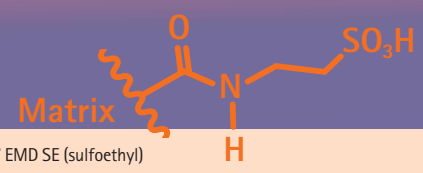
The tentacle type strong cation exchangers showed approximately double the protein binding capacity compared to other test materials. The chemical structure of the tentacle polymer is shown in Fig. 8. If it is taken into consideration that this new kind of chromatography supports have ligand densities between 70 and 120 µmol of SO3- groups per ml of packed gel (according to the manufacturer) which is not higher than for other materials this may be really a surprise. However, the surface modification of the gel particles with flexible linear polymer seems to take advantage of the increased spatial availability of the immobilized ion exchanger groups. Thus, a larger number of charges on the protein surface can be reached, or, in other words, a higher active charge density exists on the gel surface, resulting in a tighter binding of the protein. This higher active charge density may also be considered as an effect of the polyelectrolyte character of the grafted substituted polyacrylamide. The charge repulsion effect of the polyelectrolyte ligands may also shield the gel matrix and thus contribute to the high chemical stability of the gel against sodium hydroxide.

Fig 8:
Chemical structure of surface modification of tentacle-type strong cation exchangers.





Fractogel® EMD SO_3^- (sulfoisobutyl)



Fractogel® EMD SE (sulfoethyl)

Ordering information (If you are interesting in our whole range of Fractogel® products please contact us.)

Description	Catalogue No.	Contents [ml]	Particle size [µm]	Capacity [per ml gel]	Type of chromatography
Fractogel® weak cation exchanger					
Fractogel® EMD SO_3^- (M)	1.16882	100, 500, 5000	40-90	130mg Lys	strong cation
Fractogel® EMD SE Hicap (M)	1.14894	100, 500, 5000	40-90	140mg Lys	exchange chrom.
Fractogel® EMD SO_3^- (S)	1.16890	100, 500*	20-40	150mg Lys	
Fractogel® weak cation exchanger					
Fractogel® EMD COO^- (M)	1.16886	100, 500, 5000	40-90	100mg Lys	weak cation
Fractogel® EMD COO^- (S)	1.16891	100, 500*	20-40	150mg Lys	exchange chrom.

* larger quantities on request.

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