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Application of Direct Capturing in Downstream Processing of Proteins using Membrane based Chromatography

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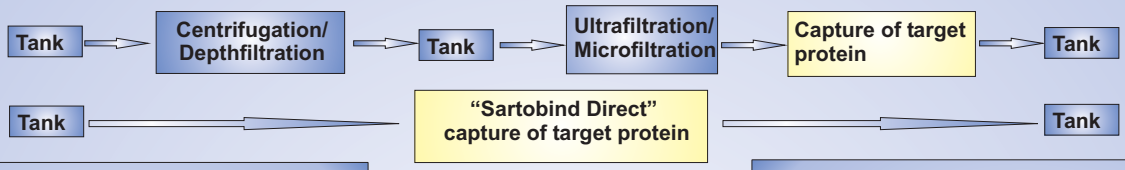
Introduction

The purification of biological proteins from complex cell culture suspensions is a cruical step in downstream processing. Traditional methods often require several steps resulting in time consuming and costly procedures. In this study a new module design based on membrane chromatography is presented and tested towards its suitability for direct capturing of proteins from cell culture suspension. This new technology combines capturing, purification and concentration of proteins in one single step. As a result, direct capturing enables a high throughput and time effective performance.



Materials and Methods

Cell line: Hybridoma cell line IV F15.23
Medium: SFM (Serum Free Medium) for hybridoma culture (Invitrogen)
Model antibody: human polyclonal IgG (IVIG = intravenous immunoglobulin)
Sartobind Protein A Direct 25 ml

Principle of Sartobind Direct

The unfiltrated cell suspension is passed over the membrane in a tangential flow mode. The target protein adsorbs on the membrane, while impurities and particels are not retained.

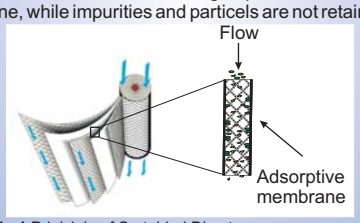


Fig.1 Priniciple of Sartobind Direct

Sartobind Direct

Materials and Methods

Cell cultivation was performed in an incubator in spinner flask (Techne) at 37°C, 25 rpm and a CO_2 atmosphere of 5%. After the cultivation, 250 ml of the cell culture suspension were spiked with a model antibody (IVIG). This solution was pumped through the, module for 40 min in recirculation mode (load) as indicated in Fig. 2 (flow rate 250 ml/min). During the loading samples were drawn every 10 min. Wash was performed in single-pass mode (flow rate 50 ml/min). Two fractions of 50 ml were collected. Four fractions of 50 ml each were collected during elution (flow rate 14 ml/min).

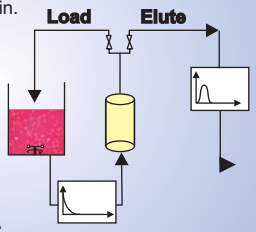


Fig.2 Experimental setup.

Parameters beeing observed:
- Optical density OD_{600}
- SDS-Page was performed for qualitative analysis
- Amount of IVIG was measured via Size-Exclusion Chromatograhy (SEC).

Results

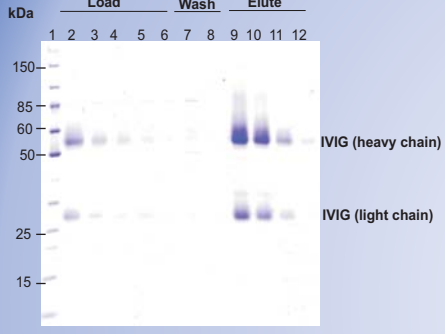


Fig.3 Purification of IVIG via Sartobind Protein A Direct from cell culture suspension (12% SDS-Gel).

The efficiency of the used Sartobind Protein A Direct 25 ml unit was tested by the separation of a model antibody (IVIG) from hybridoma cell suspension. The samples were analyzed via SDS-Page and SEC. The SDS-Page is depicted in Fig.3. The results show a continuous decrease of the light and heavy chain of IVIG in the load fractions, which means that the target molecule IVIG adsorbs to the Protein A Direct module. The elution fractions 9 and 10 show an increase of IVIG concentration compared to the load. This indicates a concentration effect of the target protein. Fig.5 summarizes the IVIG concentration of the load, wash and elution fractions.

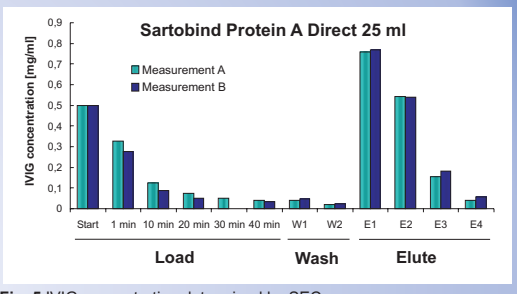


Fig. 5 IVIG concentration determined by SEC.

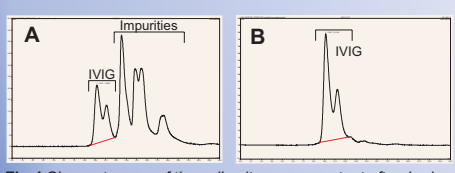


Fig.4 Chromatogram of the cell culture supernatant after 1 min of recirculation (A) and of the first elution fraction (B).

IVIG quantification was per-formed by SEC. Fig. 4 A shows the result after 1 min of recirulation. Chromatogram B shows the elution fraction without any impurities.

Tab.1 Summary of the experimental data.

	Sartobind Protein A Direct 25 ml
Membrane volume [ml]	25
Protein load [mg]	125
Protein eluted [mg]	80.6
Protein yield [%]	65
Recovery [%]	75
OD_{600} cell suspension	0.20
OD_{600} elution	0.0

Table 1 summerizes the results of the experiment. 80.6 mg of IVIG could be captured from cell culture suspension without the need of pre-filtration. The eluate was optical clear. Cells were absent in the elution fraction.

Conclusion and Outlook

A direct capture of the IVIG from cell culture suspension could be realized without any filtration or centrifugation steps. A clear and cell free eluate containing 80.6 mg of the target protein was obtained. The combination of clarification and initial purification into one single unit-operation has the potential to reduce the number of steps which are necessary for the purification of biopharmaceuticals. This study was a proof of principle. An optimization of the process towards higher product yield will be the target of future experiments. In general, this new module design seems to be suitable for direct capturing of protein from cell culture suspensions and might help to optimize downstream processes for higher cost effectiveness.