



TANGENTIAL FLOW MICROFILTRATION FOR MONOCLONAL ANTIBODY RECUPERATION OF A CELL CULTURE.

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Introduction. Initiating the purification of a monoclonal antibody (MAb) is necessary to clarify the cell culture. Higher titer of MAb is usually present in high density cell cultures however these conditions decreased cell viability and increase the presence of cellular debris, colloids and particles on the order of microns¹. One common method for the clarification is centrifugation that removes large particles, whole cells and cell debris, but the effluent still contains small contaminants to be removed to prevent clogging in further stages of the process. For this reason a secondary clarification step is required³. The aim of this study was evaluate the performance of tangential flow microfiltration (MFT) as a secondary clarification step.

Methods. In this work the primary clarification was performed with a continuous tubular-bowl centrifuge. The effluent of this operation was the starting material. For tangential flow microfiltration three filtration media were tested; polyethersulfone(PES), stabilized cellulose and PVDF. All membranes had a pore size of 0.2 microns and an area of 0.1 m². Phosphate buffer was used for diafiltration. To evaluate product recovery quantification of MAb was performed with affinity chromatography on HPLC. To evaluate the quality of clarified product turbidity was measured with a portable turbidimeter.

Results. Selection of the most adequate filtration media was based on the decrease of turbidity, recovery of product, flux and recovery of water permeability of membrane². Results are shown in table 1. The results indicate that the PVDF is the most suitable material, however polyethersulfone was more efficient for clarification and the recovery of water permeability was better than in the PVDF, for these reasons optimization of these two materials was done.

Table 1. Parameters for selection of the most adequate material.

Material	Initial Turbidity (NTU)	Final Turbidity (NTU)	Flux (LMH)	Recovery of mAb	Recovery of permeability after six uses
PES	300	13	16.5	85%	75%
PVDF	300	25	40	96%	54%
Stabilized cellulose	300	18	15	78%	80%

Behavior of flux between the two membranes was compared in function of concentration factor of retentate, the flux in PES had a slower decrease than in PVDF but in the second one the flux was higher(Figure 1). During diafiltration recovery of MAb was measured in each volume of diafiltration; PVDF needed less diafiltration volume for recovery above 90% of product, PES needed

minimum ten diafiltration volumes to reach a recovery of 90%

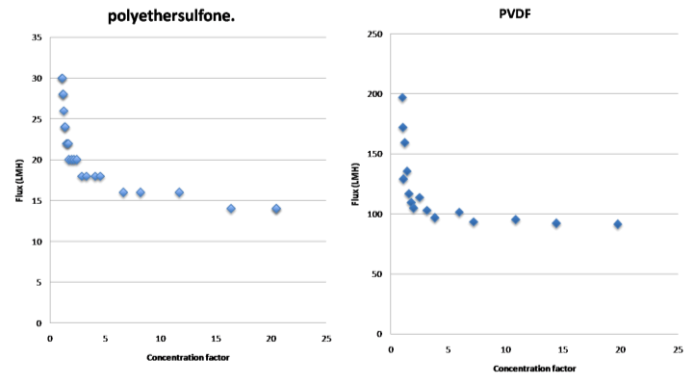


Fig.1 Difference in flux between polyethersulfone and PVDF in function of concentration factor may be related to structural differences in the filtration media

For the optimization different cross flows and transmembrane pressures (TMP) were tested. Results kept PVDF as the most suitable material, however a negative effect was seen when the cross flow and transmembrane pressure increased, indeed a turbidity increment and less recovery of product was observed under this conditions. The permeability of two membranes decreased with the uses(Figure 2), however in the PVDF product recovery did not decrease contrary to what happened with PES.

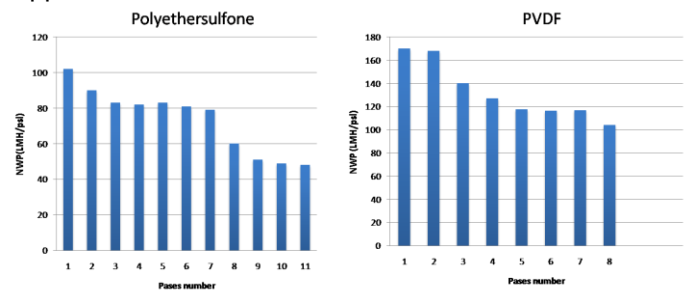


Fig.2 Behavior of the permeability in each membrane through uses

Conclusions. Filtration media used for the clarification of cell culture had big impact in flux and recovery of MAb. The efficiency of clarification is lower with higher transmembrane pressure, presumably the particles are fractionated to a sub-micron order. The MFT could be a good option to clarification of cell culture, however the required filtration area and the pump capacity could be too large, which are a limitation to application at large scale.

References.

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