TANGENTIAL FLOW MICROFILTRATION FOR HEPATITIS B SURFACE ANTIGEN PURIFICATION

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Introduction. Centrifugation scaling-up during clarification of virus like particles such as hepatitis B surface antigen is a major challenge(1). At large scale, centrifugation appears inoperative(2). Tangential flow microfiltration has been investigated as an alternative to centrifugation(3). Ultrafiltration membranes of 0.2, 0.22 and 0.45 µm were tested. The results indicate that not only the pore size has an effect through purification even the materials of membrane construction have. Careful selection of membrane was essential to maximize the recovery and to benefit the next steps in the purification.
The objective of this work was to investigate the feasibility of centrifugation replacement by tangential flow microfiltration during clarification of hepatitis B surface antigen.

Methods. Cellular disrupted broth was microfiltrated using 0.1 m^2 flat sheet Supor TFF (modified polyethersulfone, 0.2 µm, Pall), Durapore (PVDF, 0.22 µm, Millipore) and Hydrosart (stabilized cellulose, 0.2 and 0.45 µm, Sartorius). All experiments were run in a membrane cassette holder Centramate (Pall) at feeding ≤1bar. The permeate flow was restricted at ~0.3 bar. Permeate was quantified by protein assay and ELISA using Bio-Rad Protein Assay dye reagent (Bio-Rad Laboratories) and monoclonal antibodies by Abcam, respectively. Permeate was purified through the whole purification train. The best performance was tested in duplicate.

Results. Table 1 presents results for the 0.2 and 0.22 µm ultrafiltration membranes, 0.45 µm did not clarify. Concentration factor was determined according to achieved feed pressure (maximum 1 bar). The concentrate was washed until low turbidity units were observed (~3 NTU's) after that a second concentration was done. PVDF membrane showed the best performance and reproducibility referred to yield and processing time. Data was normalized to 2000 mL of initial feed volume processed.

Table 1. Performance of membranes tested during clarification of hepatitis B surface antigen cellular disrupted broth

	Modified polyether sulfone	PVDF	Stabilized cellulose	PVDF
Initial feed volume (mL)	2000	2000	2000	2000
Concentration factor	2.5	3.8	2	2
Final volume (mL)	1800	1500	2600	2400
Processing time (h)	3.6	2.2	3.1	2.9
Overall yield (%) (versus current process)	30	70	50	70

Figure 1, 2 and 3 presents performance of 0.2 and 0.22 µm ultrafiltration membranes. Figure 1 and 3 shows fast decreases of flux and turbidity. Along the concentration, turbidity has a direct correlation with product recovery. This behavior explains the poor yields obtained with modified polyethersulfone and stabilized cellulose. In the case of PVDF (figure 3), the three steps (concentration, diafiltration and 2nd concentration) can be recognized. Flux decreases slowly and also turbidity. Turbidity low values during diafiltration show the end of the step and an optimum product recovery.

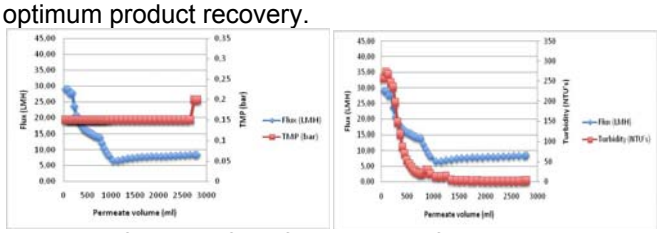

Fig.1 Performance of modified polyethersulfone membrane during clarification of Hepatitis B surface antigen

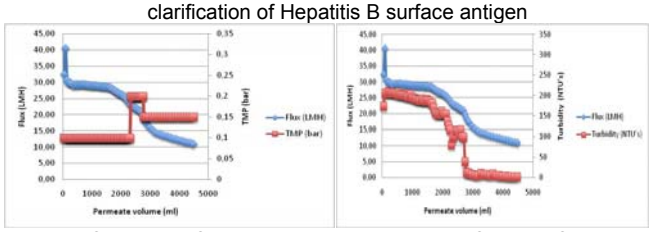

Fig.2 Performance of PVDF membrane during clarification of Hepatitis B surface antigen

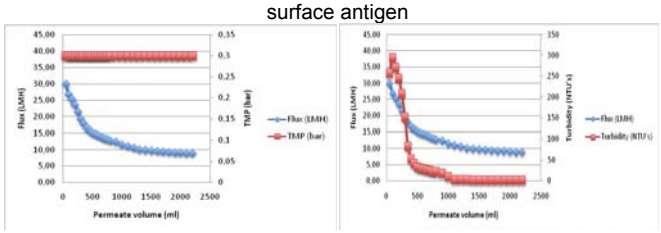

Fig.3 Performance of stabilized cellulose membrane during clarification of Hepatitis B surface antigen

Conclusions. Tangential flow microfiltration with PVDF, 0.22 µm could be used in the large-scale purification of hepatitis B surface antigen as its overall yield is acceptable because its manufacturability.

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References.

1. Wolff, Michael W and Reichl, Udo. (2011). *Expert Rev. Vaccines* 10(10), 1451–1475
2. Takahashi, Kunitoshi and Shiojiri, Susumu. (2007). *R&D Report, "Sumitomo Kagaku"*, 2007-II.
3. Gottschalk, Uwe. (2011). *Biopharm International* 24(9), 38