Selective separation of HIV-Tat protein using functionalized stacked microfiltration membranes: Enhancement of flux and recovery of protein

Saurav Datta[a], A. Nath[b], D. Bhattacharyya[a]*

a Department of Chemical and Materials Engineering, University of Kentucky
b Department of Neurology and Neuroscience, Johns Hopkins University
** Corresponding Author (saurav@uky.edu)*

Introduction

Affinity separation using functionalized microfiltration (MF) membranes provides a hydrodynamically favorable and cost-effective alternative to conventional column chromatography for the separation and purification of a specific protein from mixture of proteins. This type of separation in MF membranes is carried out by functionalizing the membrane with a suitable ligand containing specific interaction sites, and then selectively separating the target protein from mixture under convective flow.

In this research work, avidin is used as the membrane immobilized ligand for the separation of biotin-tagged Tat protein from a complex mixture of proteins. Avidin-biotin interaction is chosen because of their very strong and selective affinity for each other. Stacked membranes system is used to increase the ligand loading and hence the protein recovery.

TAT protein is a regulatory protein of HIV-1 and is potentially an excellent target for AIDS related vaccines and drug development. Tat was genetically engineered to introduce a biotin structure by bridging with a fusion protein while cloning in *E. Coli* vector. This biotin-tagged Tat was then separated from the fermentation broth (Bacterial Lysate, BL) containing other cellular proteins (>97 wt %) and impurities (cell debris, etc.) by permeating through avidin functionalized stacked MF membranes. For further medical uses Tat protein is functional in monomeric form only. The biggest challenge in Tat separation was to separate it in monomeric form as it tends to form polymers due to cysteine rich regions. It also binds with other cellular proteins.

Results and Discussions

In the initial phase of the research, it was established that high quality Tat protein (in monomeric form) containing negligible biotin impurities could be

isolated by the membrane process compared to packed bead column chromatography. SDS-PAGE and Western Blot analysis (Figure 1) were used as the analytical tools to check the quality of the purified Tat protein. However, it was also observed that to make the technique more efficient, increase in separation efficiency of Tat and permeate flux is essential. Both of them are related with the unwanted large MW proteins and other impurities present in the BL. Hence, in the latter phase of the research, a pre-filtration step (both UF and MF have been studied) was introduced to remove unwanted high molecular weight proteins and other impurities from BL feed (containing biotinylated-Tat) prior to the affinity separation.

The pre-filtration step was able to remove unwanted high molecular weight proteins and other impurities from BL feed. UF was able to remove 85±5 wt % of the total proteins (unwanted proteins) whereas, MF was able to remove 57±5 wt % of the total proteins from diluted BL supernatant. Thus, accessibility of immobilized avidin sites and hence the separation efficiency of biotinylated-Tat protein from BL was enhanced for the pre-filtered BL feed (Table 1). The quality of the purified Tat eluate obtained from the pre-filtered feed was similar to the unfiltered one, but considerably better than column chromatographically obtained Tat eluate as observed from Figure 1. Significant improvement was also observed in the flux decline behavior of the pre-filtered BL feed due to reduced fouling (Figure 2). Flux decline during affinity separation was described by simplified mathematical models. For the pre-filtered feeds fouling is assumed to occur inside the pores only (Standard Model), whereas, for the unfiltered BL feed fouling is considered both inside the pores as well as on the external surface (Combined Model is developed) of the membranes. The processing time was also reduced substantially for the pre-filtered BL feed. This also resulted in a four-fold increase in Tat recovery.

Conclusions

Superior quality of Tat protein was isolated from fermentation broth (bacterial lysate) using avidin-biotin interaction in stacked, functionalized MF membranes. The efficacy of the technique was improved by incorporating a pre-filtration step prior to affinity separation. The pre-filtration step provided the removal of unwanted high molecular proteins and other cellular impurities. Accessibility of immobilized avidin sites, and hence the separation efficiency of biotinylated-Tat protein was enhanced for the pre-filtered BL feed compared to unfiltered BL feed.

Significant improvement was also observed in the flux decline behavior of the pre-filtered BL feed due to reduced fouling. The processing time was also reduced for the pre-filtered BL feed. Membrane based separation was able to isolate superior quality of purified Tat protein compare to conventional packed-bead column chromatography.

References

[1] A. M. Hollman, D. A. Christian, P. D. Ray, D. Galey, J. Turchan, A. Nath, D. Bhattacharyya, Selective isolation and purification of Tat protein via affinity membrane separation, *Biotechnol. Prog.* **21** (2005) 451-459

[2] S. Datta, P. D. Ray, A. Nath, D. Bhattacharyya, Recognition based separation of HIV-Tat protein using avidin-biotin interaction in modified microfiltration membranes, *J. Membr. Sci.*, **280** (2006) 298-310

[3] Saurav Datta, Philip D. Ray, A. Nath, D. Bhattacharyya, Effect of Pre-Filtration on Selective Isolation of Tat Protein by Affinity Membrane Separation: Analysis of Flux, Separation Efficiency, and Processing Time, Manuscript submitted in invited edition of Separation Science and Technology

Table 1: Results of affinity separation of Tat by 4-stack avidin-immobilized Immunodyne membranes at 0.34 bar. For all three cases of affinity separation, amount of biotinylated-Tat processed = 0.55 μmoles of biotin (i.e. 4585 μg of biotinylated-Tat), maximum possible capture of biotinylated-Tat = 0.18 μmoles of biotin (i.e. 1500 μg of biotinylated-Tat), and avidin immobilized in 4-stack membranes = 0.09 μmoles (i.e. 6000 μg). UNF BL = Unfiltered BL, UF BL = Ultrafiltered BL, MF BL = Microfiltered BL

Type of BL feed	Amount of biotinylated-Tat separated (μmole of biotin)	Separation efficiency (%)
UNF BL	0.032±0.004	18±2
MF BL	0.09±0.002	50±1
UF BL	0.126±0.004	70±2

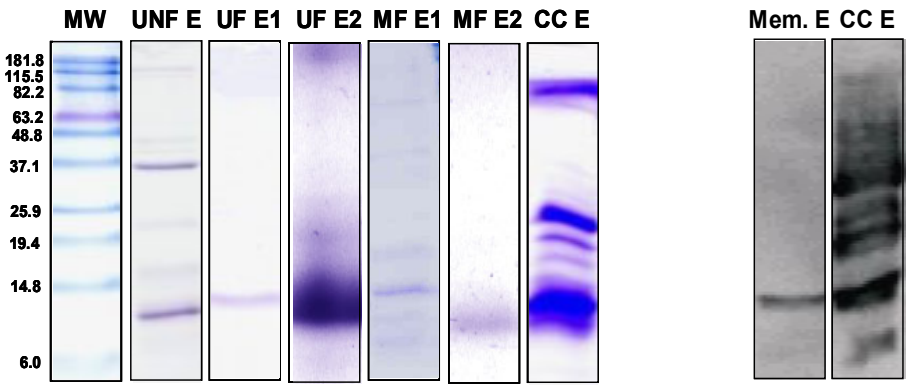

Figure 1: (a) SDS-PAGE and (b) Western Blot images of purified Tat protein eluate obtained by affinity separation. UNF E = Tat Eluate from Unfiltered BL, UF E = Tat Eluate from UF BL, MF E = Tat Eluate from MF BL, CC E = Tat Eluate from Column Chromatography, Mem. E = Tat eluate from membrane based separation, 1 and 2 denote two different experiments

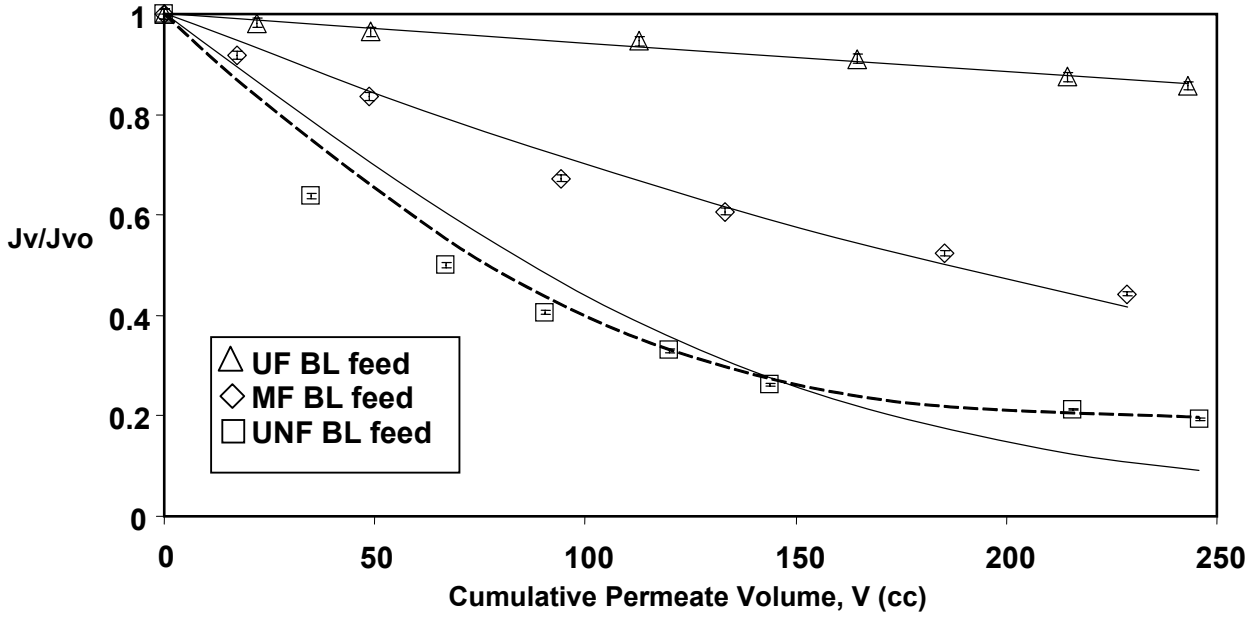

Figure 2: Normalized flux vs cumulative permeate volume for the affinity separation of different BL feeds through avidin-immobilized 4-stack Immunodyne membranes at 0.34 bar. The solid curves represent the Standard Blocking Model calculated values, whereas, the broken curve represents the Combined Model calculated values. UNF BL = Unfiltered BL, UF BL = Ultrafiltered BL, MF BL = Microfiltered BL