# Recombinant Chinese Hamster Ovary Cell Separation

Extracellular proteins were successfully separated from 600 liters of recombinant mammalian cells with high product recovery.

## Introduction

Many new generation biopharmaceuticals are being produced by mammalian cells. This bulletin describes the use of hollow fiber microporous filtration for the separation of a protein secreted by mammalian cells. The harvest has an initial concentration of about $2x10^5$ cells/ml in cell culture media. The extracellular protein product is 10 kD lymphokine similar to Interleukin-2 and is a potential cancer therapeutic. This application requires removal of cells and particulates without lysing the remaining cells, while achieving high protein recoveries. Processing time is critical because the product is thermally labile and must be kept below 10˚C.

## Process Conditions

Given the low cell concentrations, a 600-liter batch size would normally have required a filter with only 2-3 $m^2$ of membrane surface. However, since short processing times were important, two 0.2 µm rated KrosFlo® modules with 3.3 $m^2$ of membrane surface each (product no. K22M-330-01W) were installed in parallel. The estimated recirculation rate was 70 L/min. The filter inlet pressure was held steady at 5 psig. When the batch had been 30% filtered, 10 psig back pressure was applied to the filter retentate outlet, thereby raising the filter inlet pressure to about 15 psig.

Other than minor adjustments, these conditions were kept constant for the rest of the run.

Figure 1 Spectrum KrosFlo® Modules with process volumes from 3 to 1,000 liters.

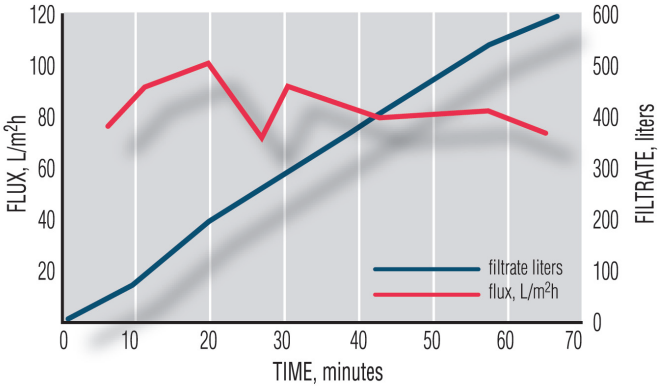


Figure 2 Flux and Throughoput vs. Time

## Results

The entire 600-liter batch was filtered in 65 minutes. Steady state flux was 85 L/$m^2$hr. Throughput-vs-time was essentially linear (figure 2). Pressures and flows were varied somewhat during the run to maximize throughput. As expected, the filters showed little flux decay, and enabled the processor to recover 94% or more of the product.

## Discussion

The protein of interest binds tightly to polysulfone membranes, but process economics require high yields. KrosFlo® cellulosic membranes exhibit superior protein passage. As single-use modules, they allow consistent membrane characteristics from run-to-run. And the risk, cost and time associated with cleaning procedures are eliminated. Spectrum modules have a variety of surface areas to allow the optimization of process times. The data presented shows that fast filtration and high recovery of heat sensitive products can be accomplished using microporous hollow fiber cross flow filtration.