DNA Removal from Bioprocess Purification Processes

Introduction

Many new biological drug products produced using recombinant DNA technology, such as monoclonal antibodies, are produced in cell culture. The host cell produces the therapeutic proteins that are often secreted outside the cell into the surrounding culture medium. Purification of the therapeutic proteins requires separation of the host cell mass from the extracellularly secreted proteins followed by an extensive series of downstream purification steps. Production of these therapeutic proteins is regulated by the Food and Drug Administration (FDA) Center for Biologics Evaluation and Research (CBER). CBER provides guidelines for biologically produced drugs including suggested limitations on final product impurities. Because therapeutic proteins such as monoclonal antibodies are produced in cell culture, impurities can result from the host cells, or cell substrates. Examples of these impurities are host cell protein and host cell DNA. In, Points to Consider in the Manufacture and Testing of Monoclonal Antibody Products for Human Use, February 28, 1997, CBER states that "It is suggested that, wherever possible, the final product contain no more than 100 pg cellular DNA per dose."

In any purification process it is desirable to remove impurities as early in the process as possible. This 3M Purification Inc. Application Brief addresses removal of DNA using cellulosic depth filters. Depth filtration is often employed for the first purification step, separation of cell mass from therapeutic proteins.

The Process

Genes coding for therapeutic proteins are inserted into host cells and fused with myeloma cells to produce hybridoma cells. These cells are then grown in fermentation vessels in order to produce the desired therapeutic proteins. Typically the therapeutic proteins are transported into the culture medium by the host cells and purification begins with separation of the cell mass from the proteins suspended in the culture medium. During this separation process host cell DNA can be released from dead or lysed cells. In addition to clarifying the cell debris from the culture fluid, it is desirable to remove as much host cell DNA as possible. Figure 1 provides a schematic of the cell separation step in the purification process.

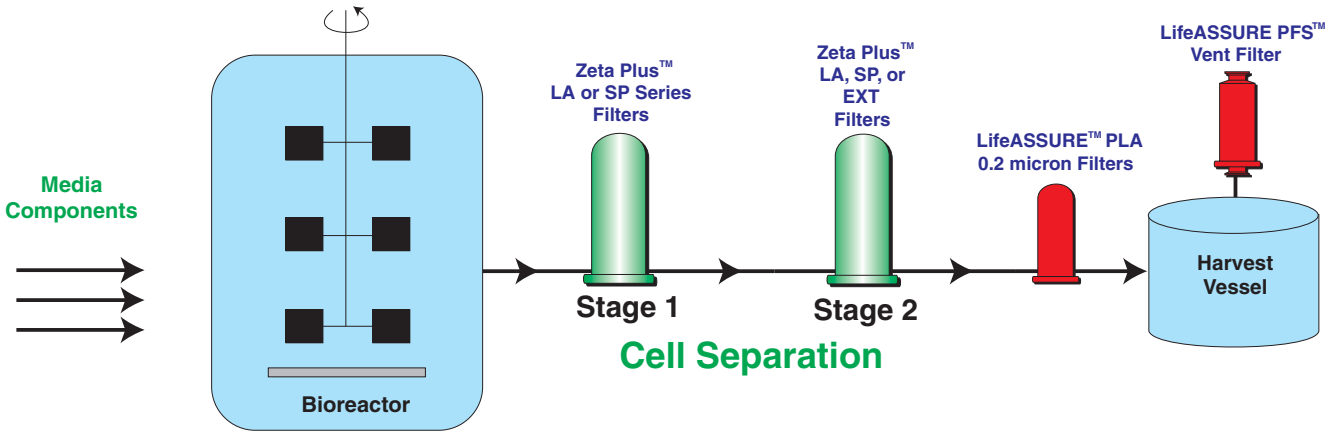

Figure 1 — Cell Separation

The Problem

The FDA through CBER has recommended that DNA in final product dosage forms should not exceed 100 pg per dose. As stated above, host cell DNA can contaminate the culture fluid as a result of release by lysed or dead cells. In, Points to Consider in the Manufacture and Testing of Monoclonal Antibody Products for Human Use, February 28, 1997, CBER states that "low cell viability may lead to high DNA levels in unprocessed bulk". The unprocessed bulk refers to the unclarifed culture fluid. In some cell culture clarification systems, centrifugation or tangential flow filtration (TFF) may be used as a primary clarification step. Both of these technologies are shear intensive and can increase cell lysis, resulting in increased host cell DNA contamination.

The presence of contaminating DNA early in a process creates the possibility for carry through during downstream purification. It is therefore important to remove as much DNA as possible during the early stages of purification. There are a number of means to remove DNA including filtration and precipitation. Precipitation involves the addition of chelating agents which, although efficiently complex DNA resulting in precipitation, represent additives to a process. Filtration, on the other hand, can remove DNA with out the need to add additional chemicals to the process.

The 3M Purification Inc. Solution

Zeta Plus™ depth filters, developed by 3M Purification Inc., contain cellulose, fiberaid, and a resin that imparts a positive charge to the filter surfaces. Examples of lenticular Zeta Plus filter cartridges are shown in Figure 2.

Zeta Plus depth filters are commonly used for initial clarification of cell culture broth and cell lysates. These filters contain significant voids volume (hence the name depth filter) which allow for accumulation of cellular debris. The filter structure is a series of interconnecting pore pathways able to retain cellular debris by mechanical entrapment. In addition to debris removal by mechanical retention, Zeta Plus filters are also able to remove particles smaller than their pore size. This capability is based on electrokinetic attraction of negatively charged particles by the positively charged filter surfaces. It was anticipated that DNA, which is a polyanionic molecule, would also be removed by Zeta Plus filters.

Figure 2 — Zeta Plus™ Filter Cartridges

In an article entitled "The Role of Charge in the Retention of DNA by Charged Cellulose-Based Depth Filters", Dorsey, et. al., BioPharm Vol. 10, January 1997, evidence was presented supporting the electrokinetic retention of DNA by positively charged Zeta Plus filters. The data in Table 1 were presented in this study.

Table 1 — Percent Capture of DNA by Charged Depth Filter Media

Zeta Plus 90SP Filter Lot Number	% Removal of DNA @	
	pH 7.4	pH 9.0
24022	99.9	29
24107	100.0	45
23443	99.8	68

The results above were per formed using calf thymus DNA at a concentration of 10 ug/ml. 100-ml challenges (7.4 L/ft^2) were performed using 47-mm filter discs and a flow rate of 14-20ml/min (1.1-1.6 L/ft^2/min). The results show significant retention of calf thymus DNA at pH 7.4 and greatly reduced DNA retention at pH 9.0, at which the positive charge of Zeta Plus is effectively eliminated. The majority of mammalian cell and bacterial lysate clarification is performed at neutral pH, where DNA retention is enhanced. When a depth filter is challenged with a solution or suspension containing DNA at a modest flux (0.5 L/ft^2/min),

DNA is initially observed to be completely removed from the solution/suspension. As binding sites within the depth filter begin to fill up, DNA is then observed to begin breaking through the filter. A complete break through curve, shown in Figure 3, is typically sigmoidal in shape as one would expect. The removal of DNA in mammalian cell culture broths is complicated by the presence of cellular debris and proteins which compete for the adsorptive sites in Zeta Plus™ filters. Based on field data, reason able DNA removal can be accomplished with a modest number of filters. A typical observed performance is approximately 1-2 logs of DNA removal at a through put of 10L culture broth per square foot of Zeta Plus depth filter medium. The recommended grades of Zeta Plus filter for best result are summarized in Table 2, below.

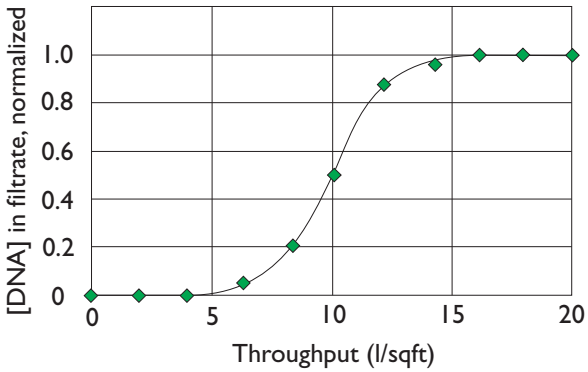

Figure 3 — Retention of DNA and Throughput

Table 2 — Recommended Grades of Zeta Plus filter for DNA Removal

Zeta Plus Type	Resin Type	Filter Grade/Nominal Porosity (µm)
Zeta Plus Activated Carbon Cartridge Zeta Plus LA Series, Zeta Plus SP Series	Tertiary amine	60/0.6 µm; 90/0.3 µm

Conclusion and Summary

The removal of DNA from cell culture broths and lysates may be required to accomplish the CBER-recommended concentration of DNA impurity of less than 100 pg/dose in therapeutic dosage forms. In many purification processes it is desirable to remove impurities as early as possible from the process. Zeta Plus depth filter medium has been extensively used in upstream processes to clarify cell culture broths and cell lysates and serves as an effective means to reduce contaminating levels of DNA. Zeta Plus filter media is available in a number of types including cellulose based and activated carbon. Both types contain a tertiary amine resin used in formulation which imparts a positive charge to the filter medium. The positive surface charge has been shown to be effective in reducing DNA by 1-2 logs. The typical throughput of culture medium or lysate observed with Zeta Plus filter systems is > 10L/ft^2 filter medium.

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