

# Cell Separation from Mammalian Suspension Cultures

## Lenticular Depth Filters As an Alternative to Crossflow Systems

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Recent years have seen an enormous increase in the production of monoclonal antibodies and recombinant proteins as potential therapeutics using mammalian cell cultures. Production of these complex molecules using cell culture systems requires considerable resources: labor, money, and time. The amount of final product can be maximized by using high-efficiency expression systems, optimizing feed media and materials, and enhancing single process steps. Therefore, a high

recovery of product after separation from culture media and purification is very important.

The first step of product purification is the separation of product from cell mass. The following parameters influence the choice of system:

- Product characteristics
- Composition of the feed media
- Economy (maximum amount of final product to be produced; costs of modules; input of time, materials, and personnel; ease of handling and maintenance; and possibility and ease of scale-up)

- Ease of validation
- Compliance with regulatory requirements

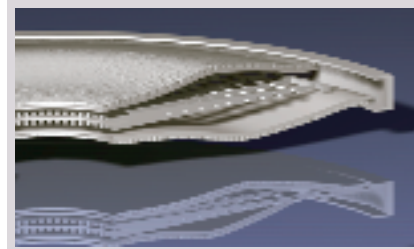
- Complexity of implementation.

Systems that fulfill most of the above requirements are depth filtration, tangential flow filtration (TFF), and centrifugation. TFF is the most commonly used system that meets laboratory, pilot, and production standards. In our study, we evaluated the use of depth filters as an alternative to TFF.

### MATERIALS AND METHODS

We used a recombinant CHO line and a dihydrofolate reductase (DHFR) expression system for product A. The cells were buffered with bicarbonate in a serum-free base medium, to which yeast extract, glucose, and insulin were

Picture 1: Section of one filter cell



added, and the cells were cultivated.

We used a recombinant CHO line that expresses glycoprotein product B, with a molecular weight of 41 kDa. The cells were cultivated in a base medium enriched with yeast extract.

**Depth Filters:** We evaluated 0.2–5.0 µm depth filters of the Zeta Plus Maximizer series as an alternative to TFF. The filter material comprises cellulose fibers, inorganic filter aids, and a binding resin that is responsible for the positive charge of the filter material. The positive charge can influence the retention quality considerably. Retention is primarily physicochemical, but the electrokinetic retention of charged media allows retention of extremely small particles and other negatively charged materials (cell debris, endotoxin, DNA, and viruses).

An additional feature is the “two media zones” that constitute the medium configuration: an open upstream zone and a tighter, qualifying

**PRODUCT FOCUS:** THERAPEUTICS FROM MAMMALIAN CELL CULTURES

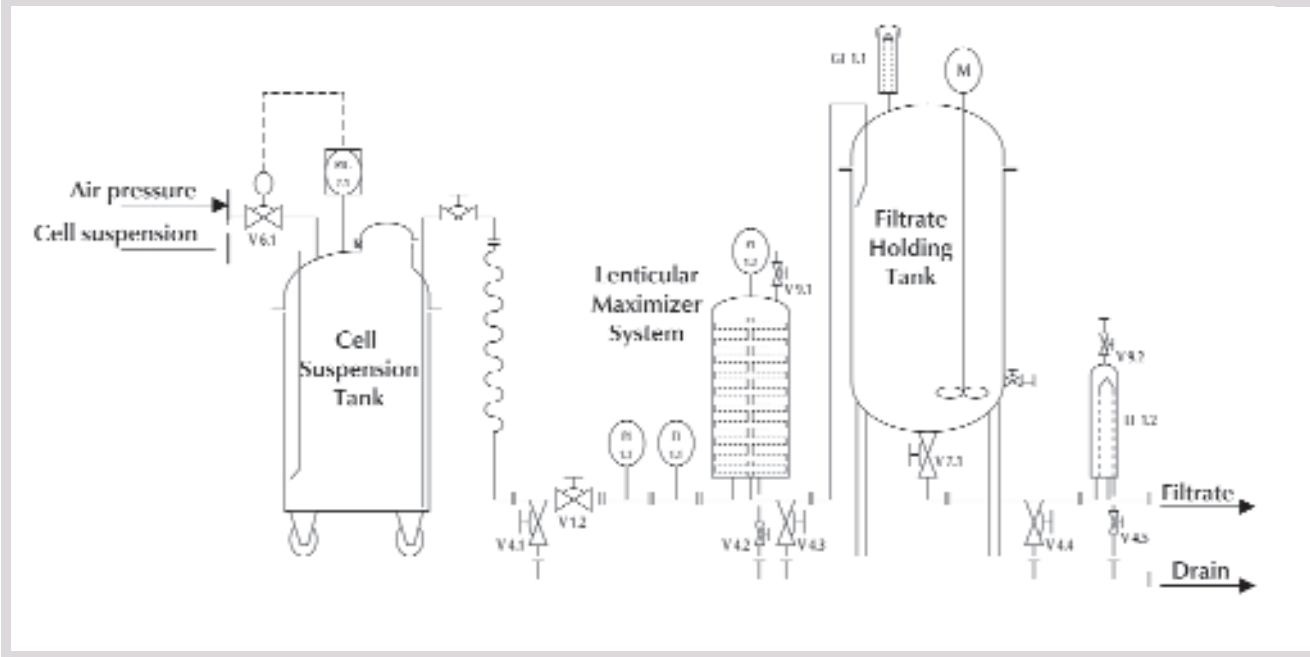
**PROCESS FOCUS:** SEPARATION (FILTRATION) AND PURIFICATION, PRODUCT RECOVERY

**WHO SHOULD READ:** PROCESS DEVELOPMENT MANAGERS, PRODUCTION

**KEYWORDS:** CELL CULTURE, FERMENTATION, RECOVERY, FILTRATION, SEPARATION AND PURIFICATION, EXPRESSION SYSTEMS, DEPTH FILTERS, TFF

**LEVEL:** BIOTECH BASICS

**Figure 1:** A schematic of the primary clarification process using Zeta Plus Lenticular filters



downstream zone. This graded filtration can significantly enhance the filter's capacity and service life.

**Method:** During fermentation of mammalian cell cultures, the product is secreted into the culture medium. The separation of product from cell mass is the first step of product extraction and purification. The main goal is to achieve the highest recovery of final product.

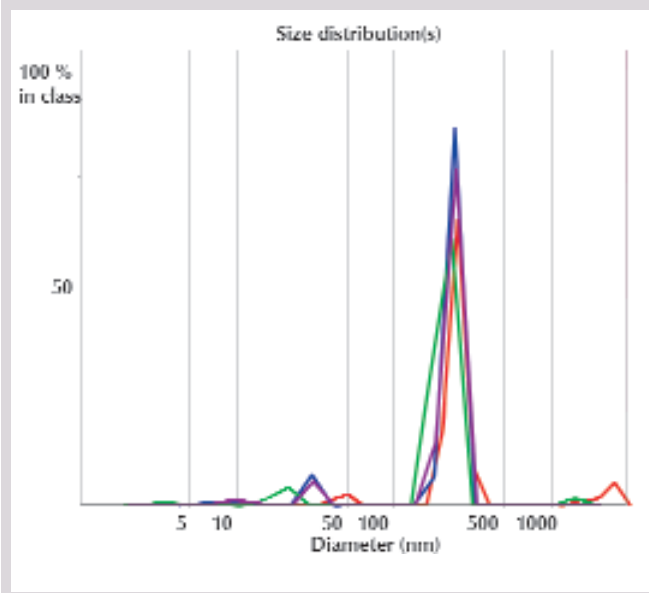
Tests with 20, 40, and 400 L of cell suspensions were carried out, and the choice of filter types and surface areas were adjusted for those volumes.

### A SCHEMATIC OF THE PRIMARY CLARIFICATION PROCESS

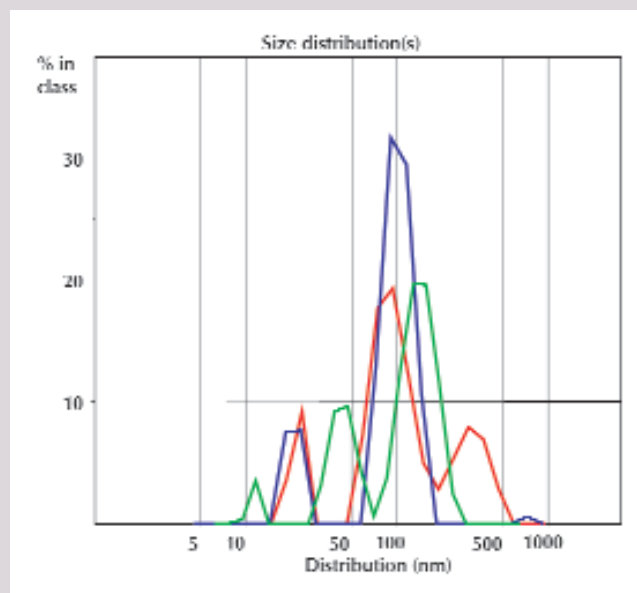
Before the process began, the filter module was prerinsed with water for injection (WFI) to reduce extractables, as recommended by the supplier. From a slightly overpressured sample tank (0.3 to 0.7 bar), the cell suspension was piped to the filter housing through a flow measuring instrument (Type COPA-XM, ABB AG, [www.abb.com](http://www.abb.com)) and pressure sensor (ED 518, range 1/2 bar, Bourdon-Haenni, [www.bourdon.com](http://www.bourdon.com)). The pressure was tested on a small

scale, and then the same conditions were transferred to production scale up to 2000 L. After the filter housing was filled and vented and the suspension filtered, the filtrate was collected in a tank. As Figure 1 shows, cell separation can be performed with minimal equipment and instrumentation compared with using TFF. The only important parameter that has to be monitored carefully and adjusted is the differential pressure across the filter. Differential pressure is monitored using experiences from Boehringer Ingelheim

**Figure 2:** Typical particle size distribution in the Zeta Plus Maximizer filtrate (one sample, three measurements)



**Figure 3:** Particle distribution after filtration with a 0.2-µm nominal rated depth filter (one sample, three measurements)



## ADVANTAGES AND DISADVANTAGES

### Benefits of Cell Separation with Depth Filters

Low material and hardware costs

Ease of use

Fast process times

High flexibility because of scale-up options (e.g., for multiproduct facilities)

Filtration with minimal shearing forces to offer cell and product protection

FDA approval of all materials

High-quality of filtrate

Space savings

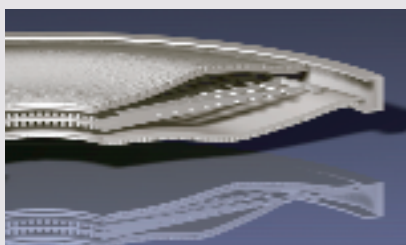
### Disadvantages of Cell Separation with Depth Filters

Necessity of monitoring differential pressure

Need to conduct economic evaluation at all scales

Need to remove and dispose of filters

Scale-up limitations



and recommendations from Cuno: As a “general rule, start with very low pressure to increase throughput, exchange filter at maximum 2.4 bar differential pressure.”

The extracted filtrate can then be passed through a clarification cartridge filter to protect the downstream equipment (purification).

### RESULTS AND EVALUATION

The systems were evaluated for recovery of product in the filtrate and reduction of particles. Product concentration in the samples was determined using an ELISA (enzyme-linked immunosorbent assay), and the quantities of particles were determined using a photon correlation spectroscopy

(PCS) system from Malvern Instruments GmbH (Table 1).

**Product Recovery:** For full-scale production of 400 L, three Zeta Plus Maximizer cartridges were used, each composed of 16 cells (partitions).

For product A, the number of cells in culture was  $1.44\text{--}5.94 \times 10^6/\text{mL}$ , with a cell viability of 14–34%. Because a high cell load may block the filter media significantly, we recommend performing scale-up tests under worst-case conditions to calculate the required filter area. The filter medium did not suffer breakage or product adsorption during this trial. Product recovery was 89–93%.

For product B, the number of cells in culture was  $1.58\text{--}2.86 \times 10^6/\text{mL}$  with a

cell viability of 16–39%. Again, exhaustion of the filter media during cell separation was not reached.


In all tests, the product recovery was above 90%. The process time including preparation and cleaning was less than two hours, which is about half the time needed with TFF.

**Particle Reduction:** Figure 2 shows the typical distribution of particles in the Zeta Plus Maximizer depth filter filtrate in the described process. Although it is a standard depth filter, efficient retention of all particles greater than  $0.3 \mu\text{m}$  can be determined.

Figure 3 shows the particle size distribution in the filtrate of a  $0.2\text{-}\mu\text{m}$  nominal rated filter cartridge (one sample, three measurements).

This demonstrates that the noncharged filter allows a greater and a larger range of particles to pass through the filter (up to  $0.9 \mu\text{m}$ ). This was not observed with Zeta Plus as shown in Figure 2.

### A RELIABLE TECHNIQUE

Cell separation using depth filters can be an economic and attractive alternative to using TFF systems. In the tests described here, the lenticular depth filter system proved to be a reliable technique. Only small product losses were observed, and superior particle retention was achieved. These tests were carried out successfully with quantities up to 400 L. 

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**Table 1:** Processing of different products and batches showing process conditions and recovery<sup>a</sup>

	Product A		Product B	
	9532	9541	18,000	18,007
Batch number.	9532	9541	18,000	18,007
Initial volume (L)	20	402	19	393
Initial suspension (mg/mL)	78.65	44.63	295	382
System type	0.16 m <sup>2</sup>	5.4 m <sup>2</sup>	0.16 m <sup>2</sup>	5.4 m <sup>2</sup>
Filtrate volume (L)	20	400	18.7	393
Filtrate volume (mL/cm <sup>2</sup> )	12.5	7.4	11.7	7.4
Content of product after filtration (mg/L)	74.95	44.63	284	360
Recovery % (titer related)	95.3	95.2	96.3	94.2

<sup>a</sup> Differential pressure: The monitored  $\Delta p$  was between 0.2 and 0.6 bar