Simplified Small-Scale Harvest of CHO Cells for mAb Analytics

Comparison of Clarification Principles: Centrifugation & Diatomaceous Earth

Michael Grauf, Bertille Lagrange, and Klaus Schöne

Monoclonal antibodies (mAbs) have been used successfully for years as therapeutic agents for different pathologies, e.g., various types of cancer and autoimmune diseases. They have become one of the main growth drivers of the pharmaceutical industry with a market size of $56.4 billion in 2012 and which is expected to reach $122.6 billion by 2019.[1]

However, antibody treatments are significantly more cost-intensive compared to drug therapies with chemically defined small molecules. This is due to the elaborate development and production processes associated with mAbs. The reduction of costs per treatment course is becoming increasingly important because of the continuous addition of new therapeutic antibodies, while the budgets of national healthcare systems are simultaneously being limited.[2]

As a result, attempts are being made both to increase the yield of antibodies per production volume and to reduce the use of capital investments through shortened development periods.[3] Cost reduction can often be achieved by continuous optimization of the individual steps.

The current study focuses on harvesting CHO cell cultures as well as on the quality of the yielded filtrate for analytical purposes. We present the use of diatomaceous earth as a filter aid within a body feed clarification.

The conventional method of harvesting consists of a centrifugation step in which particles (cells, debris, etc.) with high density or large size are separated. A subsequent microfiltration step, where suspended particles are removed from the cell culture fluid (hereinafter referred to as "centrifugation"), is carried out.

The body feed filtration method presented here enables the separation of complete cells and coarse debris by using diatomaceous earth while suspended particles are removed by microfiltration at the same time (hereinafter referred to as "DE" or "diatomaceous earth").

Sartoclear Dynamics® Lab products from **Sartorius** base their filtration principles on body feed filtration, where the filter aid diatomaceous earth (*Figure 1*) is used to form a porous cake with the suspended solids in a dead-end filter. In a conventional filtration, the cake layer solely formed by biomass is compressible and becomes rapidly impermeable, which leads to filter clogging (*Figure 2, left*). In contrast to that, the filter aid applied for body feed filtration creates a nearly incompressible filtration cake, which stays porous and prevents blockage over the complete filtration (*Figure 2, right*).

In the following, we demonstrate that the centrifugation and the body feed filtration method are comparable with regard to particle depletion and mAb recovery. Additionally, this work shows the efficiency of both methods. A heterogeneous sample pool with different types of mAb and cultivation methods was compiled to obtain a comprehensive statement.

Methods and Results

To create stable cell lines, CHO DG44 cells were transfected by electroporation and cultivated under selective conditions for three weeks followed by an amplification step with 30 nM MTX for an additional three weeks. Stable individual cell pools were then expanded, and clones were generated by FACS. Clones were analyzed for growth performance, and product concentration via fed-batch studies and genetic stability was evaluated during nine-week stability studies, including stability fed-batches (initiated at t=2 weeks, t=5 weeks, and t=9 weeks, respectively).

In 11 cultivation batches, 7 combinations of target proteins and cultivation methods were used (See *Table)*. In addition to 125-mL and 1000-mL shake flasks, 5-L stirred bioreactors (UniVessel, Sartorius) were applied. As target proteins, CHO cell lines expressing different types of antibodies or antibody-derived products (IgG1, IgG2, Fc fusion, bispecific antibody) were selected. Cell density and viability were examined with a Vi-CELL XR system from **Beckman Coulter**.

All cell culture batches were harvested after 14 days and clarified in parallel case by centrifugation and the DE method. For the clarification, sample volumes between 19–31 mL were used.

The cell culture samples (approx. 30 mL, Falcon tubes) were centrifuged for 5 min at 4,500 g and 20 °C. Subsequently, the supernatant was filtered with a syringe filter (Minisart Highflow, 16532-GUK, Sartorius) with a pore size of 0.2 µm. Depending on the sample, a varying number of filters (1–3 devices) had to be used due to blockages.

The cell culture samples were clarified with the Sartoclear

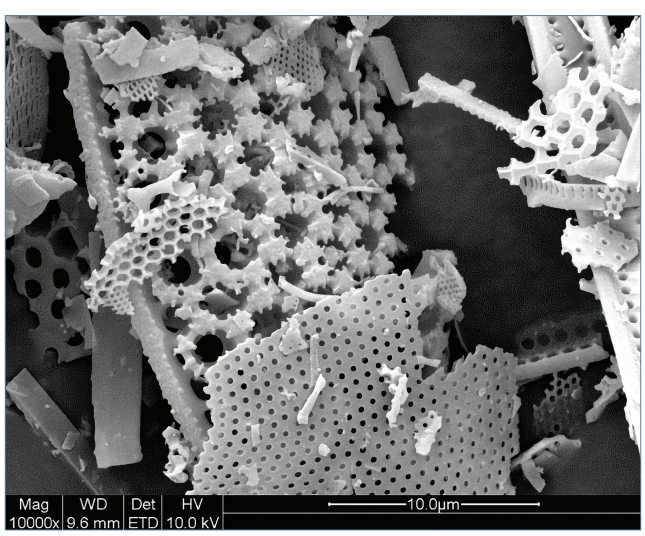

Figure 1. Scanning electron microscopy (SEM) picture of calcined diatomaceous earth (DE) with a permeability of 150–300 mDarcy.

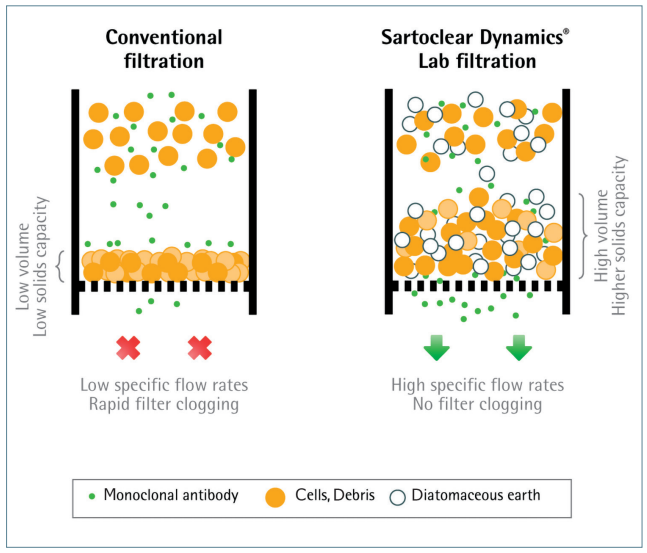

Figure 2. Principles of clarifying cell cultures by using the conventional (centrifugation) and the diatomaceous earth (DE) body-feed filtration (DE) method utilized by Sartoclear Dynamics® Lab.

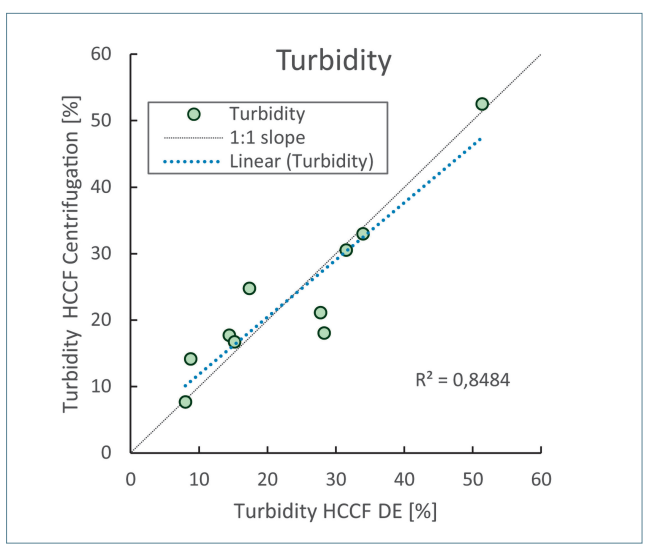

Figure 3. Turbidity of the harvested cell culture fluid (HCCF) clarified by both the centrifugation method and the diatomaceous earth (DE) method. Both clarification methods are compared regarding the turbidity of the HCCF using a scatterplot.

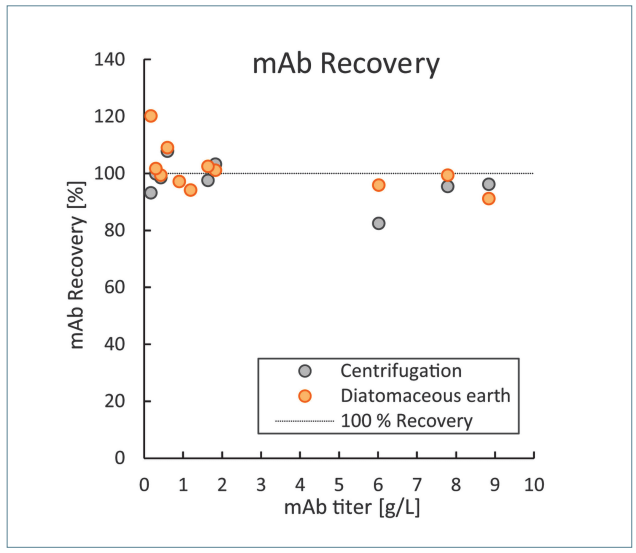

Figure 4. Recovery of mAbs in relation to their cell culture fluid titers. Samples were obtained from the filtrate after clarification by centrifugation or diatomaceous earth (DE).

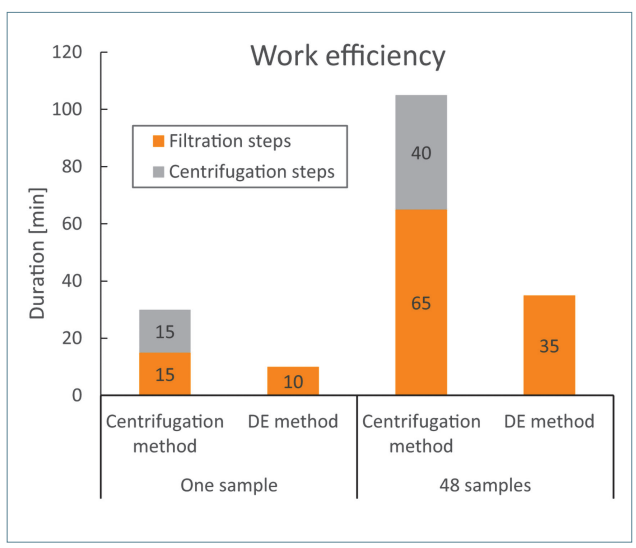

Figure 5. The duration of all centrifugation steps (including 5-minute centrifugation time and setup time) and all filtration steps were determined for the clarification methods "Centrifugation" and "DE." The duration was taken for one sample and for 48 samples.

Dynamics Lab P15. For this purpose, the cell culture suspension was taken with the syringe prefilled with DE and, after a short resuspension, filtered. The P15 is a kit comprising a 20-mL syringe prefilled with DE powder and a syringe filter equipped with a high-purity quartz microfiber prefilter and a 0.2-µm final filter made of polyethersulfone. The filling tube is useful for harvesting cell culture suspension from ambr15 bioreactors.

The centrifugation and DE methods (Sartorius) were investigated for their ability to remove cell components from the cell culture fluid (*Figure 3*). For this purpose, we determined the turbidity values before and after clarification by the TurbiCheck WL turbidimeter (**Lovibond The Tintometer**). To remain in the linear measuring range between 1 and 1,100 NTU, the samples were diluted with one-fold DPBS before turbidity measurement.

The mAb titer was determined in the cell culture fluid before and after the harvest procedure using the Octet QKe system equipped with the Protein A biosensor (ProA) from **FortéBio** without any interfering sample preparation. Due to the diversity of samples, titers were achieved in a range of 0.17 to 8.84 g/L prior to harvest (*Figure 4*).

To compare both clarification methods regarding their work efficiency, the total handling time was measured for processing one sample and for 48 samples, which were obtained from ambr15 bioreactors (*Figure 5*). The work steps were categorized as follows: "transfer from bioreactor to centrifugation tubes," "setup time for centrifugation," "centrifugation," "setup time for filtration," and "filtration" (data not shown).

Discussion

For the comparison of the conventional centrifugation method with the DE method, a heterogeneous sample pool was examined. The study investigated the following parameters: reduction of turbidity, mAb recovery, and work efficiency.

The removal of cell components, which appear as insoluble particles, were determined by nephelometric turbidity. No differences in turbidity reduction could be observed, irrespective of the turbidity of the cell culture fluid ranging between 457 and 1431 NTU prior to harvest. The turbidity in the filtrate was 8.0–51.4 NTU (*Figure 3*). These results indicate an equally effective removal of the coarse and fine particles from the cell culture suspension using both methods.

The recovery of the mAbs was investigated for various antibody types. The values vary between 91% and 109% within an acceptable range (*Figure 4*). Neither the harvest method nor the sample type, including antibody type and cultivation condition, seemed to exert an influence on product recovery. It should be mentioned, however, that even though the recovery yielded from the DE method seemed to be slightly higher, the significance of this observation has to be investigated in further experiments.

However, it has been shown that the product recovery remains unaffected in the investigated range of 0.17–8.8 g/L (*Figure 4*). Importantly, a low titer does not lead to a reduced recovery, which suggests relatively low adsorption effects.

The advantages of the body feed filtration method are reduction of handling time and equipment, especially since a centrifugation step is not required. This is particularly important for an increased number of samples, especially due to the halved handling time for the final filtration step. In comparison to the centrifugation method, the use of the body-feed filtration method using the P15 resulted in a work saving of 65% when applied to 48 samples with a volume of 15 mL (*Figure 5*).

Conclusion

In this study, we have shown the suitability of DE as a filter aid for the clarification of mAb samples dedicated for analytical purposes, in particular for cell culture samples with a turbidity below 1,431 NTU and titers in a range between 0.17–8.84 g/L. By comparing the conventional centrifugation method with the DE method prior to membrane microfiltration, we could not find any significant difference with regard to turbidity depletion and mAb recovery.

In addition, while ensuring the requirements for harvesting mammalian cell cultures—such as high recovery of mAb and sufficient reduction of the turbidity during harvest—we conclude that the DE method utilized by Sartoclear Dynamics Lab can save time and make a paramount contribution to reducing the overall workload. GEN

Acknowledgements

Special thanks go to Dr. Christoph Zehe, Dr. Dirk Müller, and Dr. Noushin Delmdahl for reviewing the manuscript and for their constructive discussion on this subject.

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Table. Results of 9-Week Stability Study

Cultivation System and mAb Product	VCC d14 [105 cells/mL]	Viability d14	Turbidity [NTU]	Titer d14 [g/L]
A1 (IgG1) 5 L UniVessel	86.9	58 %	1431	7.79
A2 (IgG1) 5 L UniVessel	155.2	78 %	1355	6.02
A3 (IgG1) 5 L UniVessel	163.6	89 %	828	8.84
B (fc-fusion protein) 25 mL in 125 mL SF	121.0	71 %	1031	0.17
C (IgG1) 25 mL in 125 mL SF	73.0	64 %	508	0.90
D1 (fc-fusion protein) 300 mL in 1 L SF	42.2	69 %	701	1.83
D2 (fc-fusion protein) 300 mL in 1 L SF	43.5	62 %	834	1.19
E (IgG2) 300 mL in 1 L SF	38.3	48 %	821	0.43
F (IgG1) 300 mL in 1 L SF	69.9	73 %	557	1.64
G (IgG1) 300 mL in 1 L SF	52.3	59 %	668	0.30
H (bispecific antibody) 300 mL in 1 L SF	46.1	69 %	670	0.60

Overview of cultivation system, target mAb, viable cell concentration (VCC), turbidity of the cell culture fluid (CCF), and mAb titer in CCF after 14 days. SF=shake flask.